THE

JOURNAL OF EXPERIMENTAL MEDICINE

THE JOURNAL OF EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D.

AND

BENJAMIN T. TERRY, M.D.

VOLUME SIXTEENTH

WITH EIGHTY-FIVE PLATES AND EIGHTY FIGURES IN THE TEXT

NEW YORK

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

1912

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

CONTENTS.

No. 1, JULY 1, 1912.

PAGE.

CECIL, RUSSELL L. The Effect of Certain Experimental Procedures on the Islands of Langerhans 1

CARREL, ALEXIS. Permanent Intubation of the Thoracic Aorta 17

BACHMANN, GEORGE. A Physiologico-Pathological Study of a Case of Heart-Block Occurring in a Dog as a Result of Natural Causes 25

Plates 1 to 7.

WADSWORTH, AUGUSTUS B. Studies on Pneumococcus Infection in Animals. First Paper 54

WADSWORTH, AUGUSTUS B. Studies on Pneumococcus Infection in Animals. Second Paper: Action of Immune Sera on Pneumococcus Infection 78

No. 2, AUGUST 1, 1912.

STEWART, HUGH A., and HARVEY, SAMUEL C. The Vasodilator and Vasoconstrictor Properties of Blood Serum and Plasma 103

Plate 8.

WOLLSTEIN, MARTHA, and MELTZER, S. J. Experimental Bronchopneumonia by Intrabronchial Insufflation 126

Plates 9 and 10.

DIXON, ROBERT L. Some Observations of the Thoracic Duct Lymph after Injection of Oil of Turpentine into the Peritoneal Cavity of the Dog 139

LEVIN, ISAAC. Changes in the Tissue Surrounding a Growing Tumor and the Significance of the "Precancerous State" 149

Plates 11 and 12.

251420

LEVIN, ISAAC. Tumor Inoculation into Organs and the Analogy between Human Cancer and the Tumors of White Mice and White Rats 155

Plates 13 to 15.

CARREL, ALEXIS. Pure Cultures of Cells 165

INGEBRIGTSEN, RAGNVALD. The Influence of Isoagglutinins on the Final Results of Homoplastic Transplantations of Arteries 169

Plates 16 and 17.

KING, JOHN H., MOYLE, R. D., and HAUPT, W. C. Studies in Glycosuria. Second Paper: Glycosuria Following Anesthesia Produced by the Intravenous Injection of Ether 178

NOGUCHI, HIDEYO. *Treponema mucosum* (New Species), a Mucin-Producing Spirochæta from Pyorrhea Alveolaris, Grown in Pure Culture 194

Plate 18.

NOGUCHI, HIDEYO. The Pure Cultivation of *Spirochæta duttoni, Spirochæta kochi, Spirochæta obermeieri,* and *Spirochæta novyi* 199

Plates 19 and 20.

NOGUCHI, HIDEYO. A Method for Cultivating *Treponema pallidum* in Fluid Media 211

LEWIS, PAUL A. The Preventive Action of Diphtheria Antitoxin in Serum-Sensitized Rabbits 216

CHURCHMAN, JOHN W. The Selective Bactericidal Action of Gentian Violet 221

Plates 21 to 31.

No. 3, SEPTEMBER 1, 1912.

MANWARING, WILFRED H. The Nature of the Bactericidal Substance in Leucocytic Extract 249

NOGUCHI, HIDEYO. Pure Cultivation of *Spirochæta phagedenis* (New Species), a Spiral Organism Found in Phagedenic Lesions on Human External Genitalia 261

Plates 32 and 33.

JOBLING, JAMES W., and STROUSE, SOLOMON. Studies on Ferment Action. II. The Extent of Leucocytic Proteolysis 269

MEARA, FRANK S., COFFEN, T. HOMER, and CREHORE, ALBERT C. A Comparison of Simultaneous Polygraph and Micrograph Tracings 280

ROBINSON, G. CANBY. A Study with the Electrocardiograph of the Mode of Death of the Human Heart 291

Plates 34 to 38.

LEVENE, P. A., and KRISTELLER, LEO. Nitrogen and Nuclein Metabolism in Gout 303

WOLLSTEIN, MARTHA. The Duration of Immune Bodies in the Blood after Antityphoid Inoculation 315

MILNE, LINDSAY S. Post-Hemorrhagic Anemia 325

Plates 39 to 42.

CRAIG, CHARLES F., and NICHOLS, HENRY J. A Study of Complement Fixation in Syphilis with Spirochæta Culture Antigens 336

PEARCE, RICHARD M. The Retention of Foreign Protein by the Kidney. A Study in Anaphylaxis 349

PEARCE, RICHARD M., AUSTIN, J. H., and KRUMBHAAR, E. B. The Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice. I. Reactions to Hemolytic Serum at Various Intervals after Splenectomy 363

PEARCE, RICHARD M., AUSTIN, J. H., and EISENBREY, A. B. The Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice. II. The Relation of Hemoglobinemia to Hemoglobinuria and Jaundice in Normal and Splenectomized Animals 375

No. 4, OCTOBER 1, 1912.

LEWIS, THOMAS. Fibrillation of the Auricles: Its Effects upon the Circulation 395

Plates 43 and 44.

INGEBRIGTSEN, RAGNVALD. Studies upon the Characteristics of Different Culture Media and Their Influence upon the Growth of Tissue Outside of the Organism 421

Plates 45 to 54.

FRASER, JOHN. The Relative Prevalence of Human and Bovine Types of Tubercle Bacilli in Bone and Joint Tuberculosis Occurring in Children 432

SPRUNT, T. P., and LUETSCHER, J. A. Acute Vascular Lesions in Mice Following Injections of Pneumococci .. 443

Plates 55 to 58.

ERLANGER, JOSEPH. Sinus Stimulation as a Factor in the Resuscitation of the Heart 452

GAY, FREDERICK P., and ROBERTSON, T. BRAILSFORD. The Antigenic Properties of Split Products of Casein 470

GAY, FREDERICK P., and ROBERTSON, T. BRAILSFORD. The Antigenic Properties of a Protein Compounded with Casein 479

JOBLING, JAMES W., and BULL, CARROLL G. Studies on Ferment Action. III. A Specific Immune Lipase 483

CRASTER, C. V. Conditions Governing the Growth of Displaced Normal Tissue 493

STEWART, G. N. Testing for Epinephrin (Adrenalin) in Blood. Comparison of Plasma and Serum 502

HANES, FREDERICK M. Lipoid Metabolism in the Developing Chick and Its Relation to Calcification 512

Plates 59 and 60.

BULLOCK, F. D., and ROHDENBURG, G. L. Cell Proliferation and Parasites in Rats 527

Plates 61 to 63.

PARK, EDWARDS A. Observations with Regard to the Action of Epinephrin on the Coronary Artery 532

Plate 64.

JANEWAY, THEODORE C., and PARK, EDWARDS A. The Question of Epinephrin in the Circulation and Its Relation to Blood Pressure 541

PARK, EDWARDS A. The Physiological Action of Epinephrin on the Bronchi 558
BASS, C. C., and JOHNS, FOSTER M. The Cultivation of Malarial Plasmodia (*Plasmodium vivax* and *Plasmodium falciparum*) *in Vitro* 567

No. 5, NOVEMBER 1, 1912.

LAMAR, RICHARD V. Chemo-Immunological Studies on Localized Infections. Fourth Paper: Experimental Pneumococcic Meningitis and Its Specific Treatment.... 581
SPRUNT, T. P., COLWELL, H. S., and HAGAN, H. J. Pigment Formation in the Liver during Autolysis and Its Relation to the Pigmentation of Hemochromatosis..... 607
OPPENHEIMER, B. S., and OPPENHEIMER, ADELE. Nerve Fibrils in the Sino-Auricular Node..... 613
Plates 65 to 67.
NOGUCHI, HIDEYO. Cultivation of *Spirochæta gallinarum*.. 620
Plates 68.
WOGLOM, WILLIAM H. The Duration and Extent of Induced Resistance toward Tumor Transplantation in Mice..... 629
COLE, RUFUS. Toxic Substances Produced by Pneumococcus. 644
DOCHEZ, A. R. The Presence of Protective Substances in Human Serum during Lobar Pneumonia 665
DOCHEZ, A. R. The Occurrence and Virulence of Pneumococci in the Circulating Blood during Lobar Pneumonia and the Susceptibility of Pneumococcus Strains to Univalent Antipneumococcus Serum 680
DOCHEZ, A. R. Coagulation Time of the Blood in Lobar Pneumonia 693
PEABODY, FRANCIS W. The Carbon Dioxide Content of the Blood in Pneumonia 701

No. 6, DECEMBER 1, 1912.

EPSTEIN, ALBERT A. A Contribution to the Study of the Chemistry of Blood Serum 719
COHN, ALFRED E. On the Differences in the Effects of Stimulation of the Two Vagus Nerves on Rate and Conduction of the Dog's Heart 732
Plates 69 to 76.

PEARCE, RICHARD M., AUSTIN, J. H., and MUSSER, JOHN H., JR. The Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice. III. The Changes in the Blood Following Splenectomy and Their Relation to the Production of Hemolytic Jaundice..... 758

KARSNER, H. T., and PEARCE, RICHARD M. The Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice. IV. A Study, by the Methods of Immunology, of the Increased Resistance of the Red Blood Corpuscles after Splenectomy 769

PEARCE, RICHARD M., and AUSTIN, J. H. The Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice. V. Changes in the Endothelial Cells of the Lymph Nodes and Liver in Splenectomized Animals Receiving Hemolytic Serum 780

Plate 77.

FOLIN, OTTO, KARSNER, HOWARD T., and DENIS, W. Nitrogen Retention in the Blood in Experimental Acute Nephritis of the Cat 789

MANDLEBAUM, F. S. A Contribution to the Pathology of Primary Splenomegaly (Gaucher Type), with the Report of an Autopsy on a Male Child Four and One Half Years of Age 797

Plates 78 to 84.

CHURCHMAN, JOHN W., and MICHAEL, W. HOWARD. The Selective Action of Gentian Violet on Closely Related Bacterial Strains 822

Plate 85.

OPIE, EUGENE L. Lymph Formation and Edema of the Liver with Experimental Nephritis Produced by Cantharidin... 831

HOWARD, C. W., and CLARK, PAUL F. Experiments on Insect Transmission of the Virus of Poliomyelitis..... 850

JOBLING, JAMES W., and STROUSE, SOLOMON. Studies on Ferment Action. V. Immunization with Proteolytic Cleavage Products of Pneumococci 860

THE EFFECT OF CERTAIN EXPERIMENTAL PROCEDURES ON THE ISLANDS OF LANGERHANS.*

By RUSSELL L. CECIL, M.D.

(*From the Pathological Laboratory of The Presbyterian Hospital, New York.*)

Although it may truthfully be said that at the present time the so-called "island theory" of diabetes is regarded with favor by the majority of those who have studied the subject, it must also be admitted that its more general acceptance has been retarded by the persistent opposition of certain investigators who maintain that the islands of Langerhans are not independent structures, but are formed by certain changes in the arrangement and properties of the acinar cells.

Of this school, Laguesse is perhaps the most zealous. In a series of exhaustive histological studies he (1) has attempted to establish his so-called "balance theory," which holds that there is a constant transformation in the pancreas of acini into islands and of islands into acini. In spite of this view, however, Laguesse believes that the islands are especially concerned in carbohydrate metabolism, and that they increase or diminish in number according to the needs of the body. Statkewitsch (2), Lewaschew (3), Dale (4), Vincent and Thompson (5) and other investigators of this school look upon the islands as merely phases of acinar life, exhausted alveoli, which may in time resume their normal appearance.

In the study of the islands the experimental procedures that have been most frequently employed are: (1) inanition experiments, (2) exhaustion of the pancreas with secretin, (3) the production of phlorizin and adrenalin diabetes, (4) ligation or partial extirpation of the pancreas.

The method last mentioned has been the most popular mode of approach, and by means of it a number of important contributions have been made to our knowledge of the islands. The present study, however, has to do with only the first three of these methods.

* Received for publication, April 12, 1912.

It has been claimed that after inanition and after exhaustion of the pancreas with secretin, there is a marked increase in both the number and size of the islands of Langerhans at the expense of the secreting parenchyma, and it has been argued from this that the islands are transitory structures without independent function. Such deductions are perfectly sound, since the islands could hardly be considered an organ of internal secretion if their existence were proved to be ephemeral. The claims, however, of these observers have been based largely on mere observation of sections; and inasmuch as the islands are known to vary greatly in number and size in apparently normal pancreases, a convincing demonstration of an increase in these properties after inanition or injection of secretin must rest on comparative numerical counts and measurements.

The work of Lazarus (6) on phlorizin and adrenalin diabetes supports the island theory. Lazarus maintains that there is a well marked hypertrophy and hyperplasia of the islands in these conditions, but his work is also open to criticism, for he gives no counts or measurements to verify this statement.

The experiments reported in this paper are repetitions of those performed by Dale, Vincent and Thompson, and Lazarus. In every case, however, the islands have been counted and measured, and the averages compared with those from controls. The counting and measuring of islands is exceedingly tedious, but the question involved is of such importance in determining the nature of the islands that the undertaking has seemed justifiable.

METHODS.

I have used dogs for the inanition and secretin experiments, while the phlorizin experiments were carried out on guinea pigs. In the dogs the sections were taken from the tail of the pancreas, about one cubic centimeter from the tip. In the phlorizin series, sections from the head, body, and tail of the pancreas were studied and an average was obtained from the three different parts.

In order that I might make use of Lane's (7) differential stain for the islands, I employed his chrome sublimate solutions for fixation. It is rather hard to obtain constantly good results with this stain, but when it is successfully used the differentiation of islands

from the secreting parenchyma is quite sharp. I have been able to demonstrate the granules in the insular cells of both dogs and guinea pigs. For counting and measuring the islands, however, the hematoxylin and eosin preparations have been invariably used. Dale's toluedin-blue and eosin stain has also been tried, but has not proved reliable.

A Leitz No. 3 objective and No. 2 ocular were employed for the counting. At this magnification the islands are easily recognized, and this is the highest magnification at which one square millimeter of the section can be seen at one time in the field. By means of a camera lucida the islands were plotted off on heavy smooth square cards which corresponded to one square millimeter of the section. Then the islands were counted and their long diameters measured. Fifty square millimeters were counted in each dog's pancreas, and approximately the same number in each guinea pig's pancreas. In order to determine the ratio of island tissue to pancreatic tissue, I followed the technique of Heiburg (8) and of Vincent and Thompson, cutting out the islands and comparing their weight with that of the remaining cardboard.

THE ISLANDS OF LANGERHANS AFTER INANITION.

A number of experimenters have studied the islands after inanition. As far back as 1894, Statkewitsch found that after starvation the acini were considerably reduced in size and were finally converted into homogeneous masses, dotted with small pyknotic nuclei. These latter structures he considered to be identical with the islands of Langerhans. Jarotsky (9), in 1899, compared the pancreases of normal white mice with those of others that had been starved to death. He found no difference in the size or number of the islands and concluded that they were independent structures.

Ssobolew (10) studied the islands in starved dogs and in dogs overfed with carbohydrates and found the insular cell protoplasm much richer in granules in the starved series than in the overfed series. He repeated Statkewitsch's inanition experiments and observed a very slight increase of islands, which he attributed to either the activity of the islands or to atrophy of the non-functionating acini. In 1904 Dale subjected toads to inanition for several months

and observed what he considered a widespread conversion of pancreatic alveoli into islands of Langerhans. Dale also found a considerable increase in the number and size of the islands of a stray cat which had apparently been without food for some time. Vincent and Thompson repeated Dale's experiments on dogs and pigeons and obtained similar results. Dale concluded from his study that the islands of Langerhans are not independent structures, but are formed by certain changes in the arrangement and properties of the acinar cells, the process constituting a reversion to the embryonic type. Vincent and Thompson maintained that when starved dogs were again subjected to a normal diet, the number and size of the islands returned to normal.

Dewitt (11) examined, measured, and counted the islands in (1) normal guinea pigs and in guinea pigs (2) after starvation, (3) after a pure carbohydrate diet, and (4) after a pure meat diet. Dewitt found the variations in size and number of the islands for the different series so slight that she considered them insignificant. She concludes that the islands are not affected by any of these procedures.

The most recent and thorough study of the pancreas after inanition is that of Bensley (12), who used guinea pigs and dogs for his experiments. Bensley made careful estimates of the number of islands after injecting neutral red into the blood-vessels of the fresh pancreas. He was unable to confirm the work of Dale so far as any increase in islands was concerned, but he demonstrated by means of his vital staining methods that the islands are in most cases in direct continuity with the ducts and acini.

In my own experiments, I first enumerated and measured the islands from the pancreases of six normal dogs, all of them on regular meat diet. The results are shown in table I. On examining this table, the most noticeable feature is the wide variation in the size and number of the islands in different pancreases. The average number of islands varied from 1.9 to 4.2 to the square millimeter; the average island diameter, from 79.1 to 99.4 micromillimeters; and the ratio of insular tissue to secreting parenchyma, from 0.8 : 100 to 1.8 : 100.

In table II I have determined the averages for all six dogs. The

TABLE I.

Starvation and Secretin Experiments.

Dog.	Duration of experiment.	Number of sq. mm. counted.	Long diameter of largest island.	Per cent. of islands with long diameters greater than 100μ.	Average long diameter of islands.	Average number of islands per sq. mm.	Ratio of island tissue to secreting parenchyma.
Control 1 . . .		50	220μ	41	97.9μ	4.2	1.8 : 100
Control 2 . . .		50	235μ	27	87.6μ	2.8	1.0 : 100
Control 3 . . .		50	225μ	39	96.3μ	2.1	0.9 : 100
Control 4 . . .		50	225μ	40	99.4μ	2.9	1.6 : 100
Control 5 . . .		50	210μ	20	79.1μ	2.7	1.0 : 100
Control 6 . . .		50	230μ	37	88.8μ	1.9	0.8 : 100
Starved dog 1	6 days	50	365μ	38	96.6μ	4.5	2.0 : 100
Starved dog 2	6 days	50	360μ	40	102.8μ	3.9	2.1 : 100
Starved dog 3	6 days	50	280μ	48	105.6μ	3.5	1.9 : 100
Starved dog 4	6 days	50	245μ	49	105.0μ	2.8	1.6 : 100
Starved dog 5	10 days	50	200μ	37	92.4μ	4.1	1.6 : 100
Starved dog 6	16 days	50	170μ	37	92.6μ	2.5	1.0 : 100
Secretin dog 1	9¾ hrs.	50	260μ	38	93.7μ	2.9	1.4 : 100
Secretin dog 2	7⅙ hrs.	50	265μ	44	100.5μ	3.3	1.5 : 100
Secretin dog 3	16½ hrs.	50	355μ	47	107.7μ	3.7	2.6 : 100
Secretin dog 4	6 1/12 hrs.	50	255μ	50	108.5μ	2.7	1.5 : 100
Secretin dog 5	10¼ hrs.	50	295μ	51	111.9μ	3.9	2.5 : 100
Secretin dog 6	16 hrs.	50	240μ	40	94.1μ	3.4	1.6 : 100

average number of islands was 2.8 to the square millimeter. The average island diameter was 91.5 micromillimeters. The ratio of island tissue to the rest of the pancreas was 1.2 : 100. According

TABLE II.

Starvation and Secretin Experiments. Comparison of Averages.

Series.	Average duration of experiment.	Average long diameter of largest island.	Average per cent. of islands over 100 μ in long diameter.	Average long diameter of islands.	Average number of islands per sq. mm.	Average ratio of island tissue to secreting parenchyma.
Control dogs.		224μ	34	91.5μ	2.8	1.2 : 100
Starved dogs.	8.4 days	270μ	41	99.2μ	3.5	1.7 : 100
Secretin dogs.	11 hrs.	278μ	45	102.7μ	3.3	1.8 : 100

to this estimate, then, the islands constitute a little more than 1 per cent. of the entire pancreas of the normal dog, a ratio, however, that is subject to wide variations.

In all these cases the acinar cells were filled with zymogen granules, which were particularly well brought out by Bensley's neutral gentian violet. The islands in a dog's pancreas are rather irregular in shape, and sometimes appear to be directly continuous with adjacent acini.

Having obtained these control estimates from normal dogs, I next undertook to determine the effect of inanition on the dog's pancreas. Six dogs were subjected to inanition for various lengths of time (table I) and estimates were made of the number and size of the islands, as in the control series.

In this series one is again impressed with the wide variations between the different cases. The number of islands per cubic millimeter varied from 2.5 to 4.5; the average island diameter, from 92.4 to 105.6 micromillimeters; the ratio of island tissue to parenchyma, from 1 : 100 to 2.1 : 100. Moreover, these variations bear no relation to the duration of inanition.

In table II the general averages obtained from the inanition series are compared with the general averages obtained from the control series. On comparing these figures, the average number of islands is found to be slightly increased in the inanition series. The difference in the average island diameter is negligible. The ratio of island tissue to parenchyma is slightly larger in the inanition than in the control series.

A histological study of the pancreas after inanition reveals no striking changes. In some places the acinar cells, as well as their nuclei, appear definitely smaller, a shrinkage which seems to be due in large part to a diminution in size of the inner granular zone. By the use of Lane's stain, however, zymogen granules are found to be present even after sixteen days of inanition. The islands are somewhat irregular in outline, but are sharply defined from the acini, and differ in no way from those in the normal dog's pancreas. In sections stained by Lane's method, the insular cells show their characteristic granulation.

The results of these experiments are in accord with those of

Jarotzky, Dewitt, and Bensley. According to my observations, the pancreas of a dog subjected to inanition does not show any active transformation of acini into islands, and the increase in the number and size of the islands after inanition is so insignificant that in consideration of the normal variations it may be ignored.

THE ISLANDS AFTER EXHAUSTION OF THE PANCREAS WITH SECRETIN.

Lewaschew was the first to study the effect of secretory activity of the pancreas on the islands of Langerhans. In 1886 he injected dogs with large doses of pilocarpin and found what he considered a well marked increase of the island tissue after this procedure. Opie (13), however, repeated these experiments, making careful counts of the islands and could find no such increase. von Hansemann (14), Ssobolew, and Vincent and Thompson have also repeated Lewaschew's experiments with negative results.

In this connection the experiments of Launoy have an important bearing. Launoy (15) showed that if the gastric juice were prevented from flowing into the duodenum, the injection of pilocarpin would not stimulate the flow of pancreatic juice. He concludes that pilocarpin is unable, of itself, to produce a pancreatic secretion.

The secretin experiments of Dale have excited considerable interest and have been widely quoted, especially by English writers, as furnishing evidence against the independent existence of the islands. Dale injected secretin at frequent intervals into the jugular vein of dogs and cats until the pancreas showed signs of exhaustion, and found a great increase in the number and size of the islands and signs of active formation of islands from acini. The same changes were found in toads injected with secretin. Dale did not count or measure the islands.

Dale's experiments have been repeated by Vincent and Thompson, who found some increase in the islands after the secretin injections, but in their experience the increase was not so marked as after inanition. Vincent and Thompson counted the islands, but do not give their figures.

The experiments of Dale have recently been repeated by Bensley with a modified technique. He substituted guinea pigs for dogs and cats, and stained the fresh pancreas *in toto* by injecting neutral

red into the blood-vessels. Bensley's secretin experiments, like his inanition experiments, were very carefully carried out, the number of islands in every case being estimated and compared with control animals. The counts in his secretin series showed a wide range of variation similar to that noted in the control series, but there was no evidence of an increased number of islands in the secretin animals. Strangely enough, the maximum number was found in one of the control animals and the minimum in one of the secretin animals. Bensley also found that the injection of secretin into toads had no influence on the islands.

I have performed Dale's secretin experiment on six dogs. In every case the secretin was prepared from the intestines of sheep or pigs according to the directions of Bayliss and Starling (16) and always produced active secretion of the pancreas. Morphin and A.C.E. were used for anesthesia. Previous to the injections, the duodenum was opened and a cannula tied into the pancreatic duct. The shortest of the six experiments lasted a little over six hours; the longest, sixteen and a half hours. The average duration was eleven hours. Although in some cases I varied both the doses of secretin and the intervals for injecting it, I was always able to maintain a constant flow of pancreatic juice. For instance, in dogs 2 and 4, I desired to maintain a maximum secretion during the entire experiment. I found that the largest flow ensued when two cubic centimeters of concentrated secretin were allowed to flow into the vein every minute. The experiment under these conditions lasted only six or seven hours. I was never able to exhaust completely the dog's pancreas, even with bleeding toward the end of the experiment. The cause of death in most of the dogs appeared to be pulmonary edema.

In the histological study of the pancreas from these dogs, the technique was similar to that for the inanition experiments and the method of counting and measuring the islands was the same. Table I shows the results obtained from the counts and measurements. In none of the secretin dogs is the number of islands per square millimeter as large as that of the first control dog. They appear in the table to be somewhat larger than the normal island, but the average island diameter in some of the control dogs is

larger than that in some of the secretin dogs. The average island diameter for the entire secretin series is 102.7 micromillimeters, about 10 per cent. greater than the average diameter for the control series (table II). The average number of islands per square millimeter was 3.3; *i. e.,* slightly larger than that for the controls. The ratio of insular tissue to parenchyma was 1.8 : 100; that is, about the same as that for the starved dogs and somewhat greater than that for the control series.

There were wide variations among the dogs subjected to the secretin experiments. The average island diameter varied from 93.7 micromillimeters (dog 1) to 111.9 micromillimeters (dog 5); the average number of islands varied from 2.7 to 3.9 per square millimeter; the ratio of island tissue to parenchyma, from 1.4 : 100 to 2.6 : 100.

If one considers the great variations in the size and number of the islands in the normal pancreas, the slight increase noted in the secretin series can hardly be given any weight; for this difference would probably have been neutralized if a larger series had been studied.

My experiments, then, have failed to demonstrate any notable increase in either the size or the number of the islands after overstimulation of the pancreas with secretin. The islands differ in no respect from those in the resting pancreas, showing, with Lane's differential stain, the same granules that are seen in the normal island. In some places the islands appear to be continuous with adjacent acini, but there is no evidence of a transition from the one form into the other. I have noted, however, that prolonged stimulation of the pancreas with secretin produces definite qualitative changes in the glandular acini. The acinar cells are noticeably shrunken, vacuolated, and granular, and their nuclei are small and pyknotic. The most striking change is the great diminution in zymogen granules. In several of my cases, they were almost entirely absent.

THE ISLANDS IN ADRENALIN AND PHLORIZIN DIABETES.

In 1907 Lazarus reported a study of the pancreas of guinea pigs in adrenalin and phlorizin diabetes. In both conditions he found

the pancreas hypertrophied and the islands increased in number and size.

Herxheimer (17) has recently repeated the adrenalin experiments, continuing the injections for five months. A careful comparison of normal guinea pigs with those injected with adrenalin showed no difference in either the number or the size of the islands.

TABLE III.

Phlorizin Experiments.

Guinea pig.	Duration of experiment.	Number of sq. mm. counted.	Diameter of largest island.	Per cent. of islands over 100μ in diameter.	Average long diameter of islands.	Average number of islands per sq. mm.	Ratio of island tissue to parenchyma.
Control 1...		50	385μ	35	105.8μ	2.2	1.4 : 100
Control 2...		46	345μ	38	107.3μ	2.0	1.3 : 100
Control 3...		57	290μ	32	96.1μ	2.5	1.5 : 100
Control 4...		52	345μ	38	110.4μ	2.5	2.0 : 100
Control 5...		59	425μ	45	103.8μ	2.4	1.8 : 100
Control 6...		50	470μ	41	108.5μ	4.2	3.2 : 100
Phlorizin guinea pig 1.	35 days	64	324μ	28	91.5μ	3.3	1.9 : 100
Phlorizin guinea pig 2.	39 days	52	234μ	32	93.9μ	1.8	1.0 : 100
Phlorizin guinea pig 3.	66 days	58	330μ	42	103.0μ	2.1	1.7 : 100
Phlorizin guinea pig 4.	103 days	52	260μ	34	83.8μ	2.2	1.2 : 100
Phlorizin guinea pig 5.	202 days	45	540μ	40	107.3μ	2.3	2.1 : 100
Phlorizin guinea pig 6.	215 days	81	420μ	38	104.9μ	2.7	2.2 : 100

In view of these negative findings of Herxheimer, it seemed to me desirable to repeat the phlorizin experiments also. Six guinea pigs were injected with one centigram of phlorizin subcutaneously every day for various periods. Guinea pig 1 received only thirty-five injections, but guinea pig 6 received two hundred and fifteen injections. In the case of guinea pig 6 the dose was gradually increased until at the time of its death it was receiving three centigrams daily.

Small sections of tissue were taken from various parts of the pancreas, were stained with hematoxylin and eosin, and the islands counted and measured in the manner already described.

In table III the results are recorded. The variations among the

individual cases in both series are just as wide as those observed in the dog's pancreas in the inanition and secretin experiments. The average island diameter in the control series varied from 96.1 to 108.5 micromillimeters; the average number of islands per square millimeter, from 2 to 4.2; the ratio of island tissue to parenchyma, from 1.3 : 100 to 3.2 : 100. In the phlorizin series, the average island diameter in the pancreas of guinea pig 4 was only 83.8 micromillimeters, while in guinea pig 5 it was 107.3 micromillimeters. The average number of islands per square millimeter was 1.8 in guinea pig 2, and 3.3 in guinea pig 1. The ratio of island tissue to parenchyma was 1 : 100 in guinea pig 2, while in guinea pig 6 it was 2.2 : 100.

In table IV the averages obtained for the entire series of phlorizinized guinea pigs are compared with those obtained in the control

TABLE IV.

Phlorizin Experiments. Comparison of Averages.

Series.	Average duration of experiment.	Average diameter of largest island.	Average per cent. of islands over 100μ in diameter.	Average long diameter of islands.	Average number of islands per sq. mm.	Average ratio of island tissue to parenchyma.
Control guinea pigs		373μ	38	105.3μ	2.6	1.8 : 100
Phlorizin guinea pigs	110 days	351μ	36	97.4μ	2.4	1.7 : 100

animals. There is surprisingly little difference in the figures. The average island diameter is eight micromillimeters greater in the control series than in the phlorizin series. This difference can be largely accounted for by the very low average diameter obtained in the case of phlorizin guinea pig 4,—only 83.8 micromillimeters. The average number of islands and the average ratio of island tissue to parenchyma are practically the same for the two series.

When sections of pancreas from the phlorizinized animals were stained by Lane's method, the granulation of both the insular and acinar cells was found to differ in no way from that observed in normal guinea pigs. The coarse zymogen granules of the acini as well as the two types of granules in the insular cells could, in either case, be well made out. There was no evidence of any kind of a transition from acinus into island or *vice versa*.

From the study of the phlorizin experiments, one is forced to the conclusion that in phlorizin diabetes, as in adrenalin diabetes, the islands of Langerhans are not affected.

DISCUSSION.

The wide variation in the number and size of the islands of Langerhans in normal pancreases is probably in large part responsible for the erroneous conclusions which have been reached by Dale, Vincent and Thompson, Lazarus, and others. Such mistakes were all the easier to make when no comparative counts and measurements were made. Bensley points out the fact that areas of post-mortem degeneration fail to take up toluidin-blue, and he suggests that Dale may have mistaken these areas for large islands. It is undoubtedly true that prolonged stimulation of the pancreas with secretin produces a certain amount of self-digestion, caused probably by the greatly increased amount of pancreatic juice; and in some of Dale's photographs (particularly figure 5) the areas displayed are very suggestive of autolysis.

Dale speaks of "exhausting" the pancreas with secretin, but admits that exhaustion of the mammalian pancreas is almost impossible. I have found it so, even after the injection of active secretin for sixteen hours. I can see no reason why it should become completely exhausted as long as the animal is alive, and blood and secretin continue to circulate through it. The theories of Laguesse and of Dale which assume that acini may be rapidly converted into islands, or islands into acini, have for their support the pictures sometimes seen of islands in continuity with acini. But, as Bensley says, in order to prove this point, it is necessary "to show not only the phases of the disappearance of the specific elements at one end of the series, but also the appearance of the specific elements at the other end." The same writer argues that it is reasonable to suppose that this change could not go on with a rapidity too great to permit the discovery of intermediate phases, because the anatomical structure of an island necessitates a considerable degree of anatomical rearrangement in constructing islands out of acini. As a matter of fact, however, these intermediate phases are lacking and by the use of Lane's granule stain, it can

be shown that many of the structures which have the appearance of transitional forms are entirely free from the characteristic granulations of island cells.

These continuations between acini and islands can be explained in a much more satisfactory way. By the cutting of serial sections, it has been shown by Laguesse, Weichselbaum and Kyrle (18), Cecil (19), and Bensley that the islands in both health and disease may often be directly connected with the ducts or acini, from which they have developed. Bensley's work on this subject is one of the most important contributions to our knowledge of the islands that has appeared in recent times, and in conjunction with certain new pathological data, it would seem to have established beyond doubt the anatomical independence of the islands of Langerhans. By means of new vital staining methods he has been able to demonstrate an intricate anastomosing network of small tubules, which are connected on the one hand with the pancreatic ducts, and on the other with acini and the islands of Langerhans. In rare cases only does the lumen of the tubule acually penetrate the substance of the island, so that, notwithstanding its direct connection with the tubule, the island is unquestionably a ductless gland. One of the most important points brought out by Bensley is the fact that the majority of the islands are connected in this manner with the ducts.

As far as the phlorizin experiments are concerned, my results serve to substantiate still further the prevalent belief that phlorizin diabetes is not of pancreatic origin.

CONCLUSIONS.

Although the results of this study have been of a negative character, the conclusions that may be drawn from it have an important bearing on the true anatomical character of the islands.

1. Neither inanition[1] nor the prolonged injection of secretin has any noteworthy effect upon the number, size, or structure of the islands of Langerhans in the dog's pancreas.

[1] The inanition experiments were carried out partly at The Rockefeller Institute for Medical Research, and partly at the Cornell University Medical School. For the privileges of these institutions I am indebted to Dr. Simon Flexner and to Dr. James Ewing.

2. The islands of Langerhans in the guinea pig's pancreas are in no way altered in phlorizin diabetes.

3. The islands of Langerhans are not formed out of exhausted or degenerated acini, but develop from the ducts or acini with which they are often in direct continuity.

PROTOCOLS.

Control Dogs 1 to 6.—Normal dogs on normal diet. Anesthetized with ether. The pancreas in every case appeared normal. Small bits of pancreas were preserved in Lane's chrome sublimate solutions.

Starved Dog 1.—Mongrel male; weight 9,000 gm. Subjected to inanition for 6 days and then bled to death. Stomach and intestines empty. Pancreas firm and pale.

Starved Dog 2.—Fox terrier, female; record of weight lost. Subjected to inanition for 6 days, then killed by ether. At autopsy, fairly well nourished. Stomach and small intestine empty. Small amount of feces in rectum. Pancreas pale.

Starved Dog 3.—Mongrel, female; record of weight lost. Subjected to inanition for 6 days, then killed by ether. Fairly well nourished animal. At autopsy, the stomach contained a little brownish mucus with shreds of hay in it. Small intestine empty. Small quantity of feces in rectum.

Starved Dog 4.—Mongrel, male; record of weight lost. Subjected to inanition for 6 days, then killed by ether. At autopsy, the stomach contained a little hay and mucus. The small intestine contained a tapeworm. There was a small quantity of feces in the rectum.

Starved Dog 5.—Male; weight 12,500 gm. Subjected to inanition for 10 days, then bled to death. Weight at time of death, 10,200 gm. Stomach and intestines empty. Pancreas normal.

Starved Dog 6.—Male; weight 10,700 gm. Subjected to inanition for 16 days and then bled to death. Weight at time of death, 8,250 gm. Stomach and intestines empty. Pancreas normal.

Secretin Dog 1.—Male; weight 4,944 gm. The first injection of secretin produced active secretion from the pancreatic duct, seven or eight drops to the minute. After six hours the responses to these injections were much less marked, only three or four drops to the minute being counted. Duration of experiment, 9¾ hours, the dog receiving 8 c.c. of secretin every five minutes except during the last two hours of the experiment, when he received 5 c.c. of secretin every three minutes. During the last two hours the dog was bled several times. The pancreas responded to secretin to the last.

Secretin Dog 2.—Female; weight 9,300 gm. During the first hour and a half of the experiment, the dog received 1 c.c. of secretin every minute. After that she received 2 c.c. every minute. There was a flow of two or three drops of pancreatic juice to the minute. Duration of experiment, 7⅛ hours. The dog was bled toward the end of the experiment. Although the secretion was not so active toward the end, there were no signs of pancreatic exhaustion.

Secretin Dog 3.—Male; weight 6,420 gm. During the first thirteen hours

of the experiment, the dog received 5 c.c. of secretin every five minutes. After that he received 1 c.c. every minute. The pancreas responded actively, especially to the latter method of administration. Duration of experiment, 16½ hours. The dog died of pulmonary edema. He was not bled at any time during the experiment. The flow of pancreatic juice continued to the end.

Secretin Dog 4.—Female; weight 6,550 gm. The secretin was more concentrated than that used in previous experiments. During the first two hours, the dog received 1 c.c. of secretin every minute. The pancreas responded well (five to six drops of juice a minute). During the remainder of the experiment, the dog received 2 c.c. of secretin every minute. Duration of experiment, 6½ hours. Toward the end the flow of pancreatic juice diminished to one drop a minute, but there was never complete exhaustion. The cause of death was apparently pulmonary edema. The dog was not bled at any time during the experiment.

Secretin Dog 5.—Male; weight 21,000 gm. The dog received 10 c.c. of secretin intravenously every five minutes. The flow of pancreatic juice was fairly active. Duration of experiment, 10½ hours. No signs of pancreatic exhaustion. The animal stopped breathing rather suddenly, after receiving a small dose of morphin.

Secretin Dog 6.—Mongrel, male; weight 8,500 gm. The dog received 5 c.c. of secretin every five minutes for 16 hours. In this experiment the efficacy of the secretin was tested on a control dog, but in the case of the subject for the experiment the abdomen was not opened. The cause of death was respiratory failure. At autopsy there were small fat necroses scattered over the pancreas which was dark red and edematous.

Control Guinea Pigs 1 to 6.—Normal guinea pigs fed on vegetable diet. The pancreas in every case appeared normal macroscopically and microscopically.

Phlorizin Guinea Pig 1.—Weight 210 gm. Daily subcutaneous injections of phlorizin for 35 days. Dose, 1 cg. of phlorizin (dissolved in warm water). Loss of weight, 40 gm. Urine gave a positive Fehling test for glucose. The fermentation test indicated 0.2 to 0.4 per cent. glucose. At autopsy the pancreas appeared normal.

Phlorizin Guinea Pig 2.—Weight 260 gm. Daily subcutaneous injections of phlorizin for 39 days. Dose, 1 cg. Loss of weight, 110 gm. Urine gave a positive Fehling test for glucose (0.2 to 0.4 per cent.). At autopsy the pancreas appeared normal.

Phlorizin Guinea Pig 3.—Weight 200 gm. Daily subcutaneous injections of phlorizin for 66 days. Dose, 1 cg. The weight remained about stationary. Urine gave a positive Fehling test (0.2 to 0.4 per cent.). At autopsy the pancreas showed no gross changes.

Phlorizin Guinea Pig 4.—Weight 335 gm. Daily subcutaneous injections of phlorizin for 103 days. Dose, 1 cg. Slight gain in weight. Urine for twenty-four hours contained from 0.25 to 0.48 gm. of glucose. At autopsy the pancreas was rather large but otherwise normal.

Phlorizin Guinea Pig 5.—Weight 345 gm. Daily subcutaneous injections of phlorizin for 202 days. Dose, 1 cg. Moderate gain in weight. Twenty-four hour specimens of urine examined from time to time contained from 0.18 to 0.9 gm. of glucose. At autopsy the pancreas was infiltrated with fat.

Phlorizin Guinea Pig 6.—Weight 445 gm. Daily subcutaneous injections of phlorizin for 215 days. The dose was 1 cg. during the first 120 days; during the following 47 days, 2 cg.; and for the last 12 days, 3 cg. Moderate gain in weight. Twenty-four hour specimens of urine contained from 0.24 to 1.2 gm. of glucose. At autopsy the pancreas was infiltrated with fat.

BIBLIOGRAPHY.

1. Laguesse, E., *Arch. d'anat. micr.*, 1909–10, xi, 1.
2. Statkewitsch, P., *Arch. f. exper. Path. u. Pharmakol.*, 1894, xxxiii, 453.
3. Lewaschew, S. W., *Arch. f. mikr. Anat.*, 1886, xxvi, 453.
4. Dale, H. H., *Phil. Tr. Royal Soc. London, Series B*, 1904, cxcvii, 25.
5. Vincent, S., and Thompson, F. D., *Internat. Monatschr. f. Anat. u. Physiol.*, 1907, xxiv, 61.
6. Lazarus, P., *München. med. Wchnschr.*, 1907, liv, 2222.
7. Lane, M. A., *Am. Jour. Anat.*, 1907–08, vii, 409.
8. Heiburg, K. A., *Anat. Anz.*, 1906, xxix, 49.
9. Jarotzky, A. J., *Virchows Arch. f. path. Anat.*, 1899, clvi, 409.
10. Ssobolew, L. W., *Centralbl. f. allg. Path. u. path. Anat.*, 1900, xi, 202; *Virchows Arch. f. path. Anat.*, 1902, clxviii, 91.
11. Dewitt, L. M., *Jour. Exper. Med.*, 1906, viii, 193.
12. Bensley, R. R., *Am. Jour. Anat.*, 1911, xii, 297.
13. Opie, E. L., *Bull. Johns Hopkins Hosp.*, 1900, xi, 205.
14. von Hansemann, *Verhandl. d. deutsch. path. Gesellsch.*, 1901, iv, 187.
15. Launoy, L., *Arch. internat. de physiol.*, 1905–06, iii, 62.
16. Bayliss, W. M., and Starling, E. H., *Jour. Physiol.*, 1902, xxviii, 325.
17. Herxheimer, G., *Verhandl. d. deutsch. path. Gesellsch.*, 1909, xiii, 276.
18. Weichselbaum, A., and Kyrle, J., *Arch. f. mikr. Anat.*, 1909, lxxiv, 223.
19. Cecil, R. L., *Jour. Exper. Med.*, 1911, xiv, 500.

PERMANENT INTUBATION OF THE THORACIC AORTA.*

By ALEXIS CARREL, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

In a previous article[1] it was shown that a segment of vein could be grafted successfully on the thoracic aorta. While this procedure might prove to be the ideal treatment of aneurysms on peripheral arteries, the extirpation of the aneurysmal sac and the transplantation of a vascular segment upon the thoracic aorta would be complicated and dangerous. I have attempted, therefore, to determine the proper technique for intubating the aorta, as this operation is simpler and may prove more satisfactory than grafting.

The idea of introducing a tube into the lumen of a vessel is not original. In fact paraffined glass tubes are used in all physiological laboratories for securing an artificial circulation of blood, and the same method has been applied successfully by Brewer to the transfusion of blood in human beings. Some years ago Abbe tried to effect a permanent anastomosis between the cut ends of an artery by means of a glass tube. He presented at the Academy of Medicine of New York a cat in which he had cut the abdominal aorta and had united the ends of the vessel by a glass tube. In other cases coagulation of the blood took place after a short time. It appeared therefore that even if a foreign body could be put temporarily in a vessel without accident, thrombosis would occur sooner or later.

Nevertheless, it was conceivable that thrombosis was preventable, and that the conditions under which a foreign body could be used for repairing a vessel might be ascertained. I extirpated, therefore, part of the anterior wall of the abdominal aorta of a dog, and replaced it by a piece of rubber covered with vaselin. Fifteen months after the operation, the circulation was still normal. The

* Received for publication, April 4, 1912.

[1] Carrel, *Jour. Exper. Med.*, 1912, xv, 389.

vessel wall was intact inside and outside of the rubber. It was found also that the circulation could take place for six days through a glass tube lying free in the abdominal aorta. Thrombosis occurred subsequently owing to displacement of the tube. Encouraged by these results, I operated upon a number of dogs with the purpose of learning the precautions that must be taken in order to make the intubation successful. In all instances the operations were performed on the thoracic aorta of medium-sized dogs. The thoracic cavity was opened under ether anesthesia by the method of Meltzer and Auer, and a glass or metal tube was inserted into the lumen of the descending aorta.

The animal was etherized and the hair on the left side of the thorax was removed by sodium sulphid. The skin was washed with alcohol and covered with 10 per cent. tincture of iodin. Then a catheter was introduced into the trachea and connected with a Meltzer and Auer apparatus which consisted of a foot bellows, an ether bottle, and a small manometer. Next the thoracic cavity was opened by an incision passing through either the eighth or the fourth intercostal space. As soon as the pleura was opened, silk compresses sterilized in vaselin were introduced in order to protect the left lung, and to wall off the pleural cavity. Then a retractor of Gosset was placed between the ribs and opened as much as was necessary. Another silk compress was introduced in the cavity in order to circumscribe completely the operating field. Since sponging irritates the pleura, it is very important that no blood should escape into the cavity.

The pleura was cut along the portion of the aorta that had to be intubated, but the aorta itself was not dissected, and its collateral branches were not ligated. Two ligatures of soft but heavy silk thread were passed around the aorta approximately at the points where the ends of the tube were to be fixed. A few centimeters above and below these two ligatures, two elastic forceps were kept ready for clamping the vessel. The elastic blades of the forceps were applied directly to the arterial wall, no rubber being interposed.

The wall of the aorta was then cut longitudinally. The edges of the incision were caught with two forceps and the tube was introduced into the lumen of the aorta. The tubes were made of glass

or aluminum. They were generally nine or ten millimeters in diameter and forty-five millimeters long. As the dogs were of various sizes, the tubes either distended the vessels, fitted them exactly, or were smaller than the caliber of the aortas. The tubes were straight, but their edges were slightly everted. The glass tubes were crude, their edges being often irregular and sharp. Before being used, the tubes were paraffined. As soon as the tube was inserted into the lumen of the vessel, the silk threads that had been put around the aorta previously, were tied. The ligature should be tight enough to prevent the tube from slipping, but not tight enough to cut the wall of the artery. This wall is easily injured.

The clamps were removed and the circulation was reëstablished through the intubated segment. Generally there was no hemorrhage from the incision through the aortic wall, and in some cases the opening was not closed. In others it was sutured with fine silk. It is safer to close the opening carefully even if there be no bleeding. The pleura was then sutured along the aorta. The silk compresses were removed and the ribs were approximated by two silk ligatures. Before closing the thoracic cavity, slight compression was made on the trachea in order that the lungs might regain their normal size. The muscles and then the skin were united by continuous sutures.

Ten experiments were made. In one the introduction of a tube into a comparatively small aorta resulted in tearing the wall, and the animal was killed immediately by chloroform. The nine other cases will be briefly described.

Experiment 1.—Medium-sized bull terrier, No. 729. On January 18, 1912, 10:16 A. M., a transverse thoracotomy was made in the tenth intercostal space, under ether anesthesia by the Meltzer and Auer method; the lower part of the thoracic aorta was dissected. At 10:50 temporary hemostasis was caused by the two pairs of elastic jawed forceps. The aorta was incised and the paraffined glass tube 45 mm. in length and 9 mm. in diameter was introduced. The tube was fixed by an upper ligature of rubber and by a lower ligature of silk, both being very tight. The circulation was reëstablished at 10:56. The incision in the arterial wall was not sutured, and the thoracic cavity was closed at 11:18. After the operation the femoral pulse was normal and the animal remained in excellent condition until January 26, when it appeared sick and no pulsation was felt in the right femoral artery. On January 27 the animal was again in good condition, but on January 29, at 10 A. M. it died suddenly.

Autopsy.—The thoracic cavity was filled with blood. The ligatures, which had been too tight, had cut the arterial wall and a sudden hemorrhage resulted.

The tube was in the aorta. No paraffin or fibrin could be seen on its wall. But on the wall of the aorta near the upper end of the tube, there was a slight laceration covered by a thin deposit of fibrin. There was no clot in the iliac arteries.

Experiment 2.—Black and white female dog, No. 769. On January 26, 1912, at 10:20 A. M., a transverse thoracotomy was made in the eighth intercostal space, under ether anesthesia by the Meltzer-Auer method. At 10:28 temporary hemostasis of the middle part of the descending aorta was caused. This was followed by a longitudinal incision of the vessel and the introduction of a paraffined glass tube 45 mm. in length and 9 mm. in diameter. The tube was fixed at each end by a ligature of heavy surgical silk, but the aortic incision was not sutured. The circulation was reëstablished at 10:36, and at 10:55 the operation was ended. The animal remained normal until February 5, but on February 5, at 4 P. M. the pulsations of the femoral arteries disappeared, and the dog died shortly afterwards.

Autopsy.—There was no hemorrhage in the thoracic cavity, but a large clot was found adherent to the non-sutured incision of the aortic wall. Longitudinal incision of the aorta revealed the fact that the threads had not cut the arterial wall. The upper end of the tube, which was too sharp, had caused a slight laceration of the vessel wall. The tube was lined with fibrin, except in its lower part. The upper part of the aorta was open, but the lower part was completely obliterated by a thrombus. This had been produced by the fibrin in the lower end of the tube becoming loose and being swept by the blood stream into the lumen of the aorta.

Experiment 3.—Young, brown female dog, No. 798. On January 30, 1912, at 10:21 a thoracotomy was made through the eighth intercostal space under ether anesthesia by the Meltzer-Auer method. At 10:35 temporary hemostasis was caused in the middle part of the descending aorta. The wall was incised, and a paraffined glass tube was introduced. The tube was 45 mm. long and 9 mm. wide. Its caliber proved to be smaller than that of the aorta, and the edge of its upper end was slightly everted. A ligature of heavy silk was put around the aorta at the level of the upper end of the tube, but the lower end was not ligated. The tube fitted the aorta exactly, and the incision was not sutured. At 10:38 the circulation was reëstablished, and at 11:05 the thoracic cavity was closed and the operation ended. The animal remained normal.

Experiment 4.—Young, white female dog. On February 6, 1912, at 10:31 under ether anesthesia by the Meltzer-Auer method, a thoracotomy was made through the eighth intercostal space on the left side of the animal. At 10:47 a thin-walled paraffined glass tube 40 mm. long and 9 mm. wide was introduced into the descending aorta by the usual procedure. The tube was fixed by two heavy ligatures which were tied loosely. The circulation was reëstablished at 10:52, and at 11:10 the operation was ended. The animal remained normal for eight days, but died suddenly on February 14.

Autopsy.—The thoracic cavity was filled with blood, for the glass tube had broken in the middle, and hemorrhage had taken place through the aortic incision. There was no clot in the tube or in the aorta, and no paraffin or fibrin was seen on the glass wall. There was no ulceration of the aorta at the ends of the tube. In fact the aortic wall was in excellent condition.

Experiment 5.—White and brown female dog, No. 893. On February 9, 1912, at 10:22 A. M., a left thoracotomy was made through the eighth intercostal space under ether anesthesia by the Meltzer-Auer method. At 10:36 A. M. during temporary hemostasis a paraffined glass tube was introduced, and was fixed by two heavy silk ligatures, although the tube seemed too narrow for the aorta. The circulation was reëstablished at 10:40. The aortic incision was then sutured. At 10:49 the thoracic cavity was closed, and at 11:02 the operation was ended. The animal remained in good health for twenty-three days, but on April 4, while the dog was running, its posterior limbs became suddenly paralyzed. After about three hours, it was again in normal condition. On April 7, 1912, it was found dead in its cage.

Autopsy.—The aorta was obliterated just below the lower end of the tube by a fibrinous clot. The thrombus originated apparently from a laceration produced in the aortic wall by the lower end of the tube, which was very sharp. The upper end had caused no laceration. The lumen of the tube was entirely free.

Experiment 6.—Black and white, long haired, male dog, No. 920. On February 13, 1912, at 10:25 A. M., under ether anesthesia by the Meltzer-Auer method, the thoracic cavity was opened and the descending aorta was prepared in the usual way. At 10:36 during temporary hemostasis, a paraffined aluminum tube 45 mm. in length by 10 mm. in diameter was introduced into the descending aorta. The tube was fixed by heavy silk ligatures but not very tightly. The caliber of the tube was markedly smaller than that of the aorta. The aortic incision was then sutured. The circulation was reëstablished at 10:39, and the thoracic cavity was closed and the operation ended at 11:02. The dog remained in excellent condition. On March 25, the animal was apparently in good health. On March 26 it died rather suddenly.

Autopsy.—The pleural cavity was distended by a clear serosanguineous fluid. The intubated portion of the aorta was surrounded by a large tumor which was composed of granulation tissue. The tube was corroded and partially occluded by a clot of fibrin.

Experiment 7.—Brown female spaniel, No. 940. On February 16, 1912, at 10.13 A. M., under ether anesthesia by the Meltzer-Auer method, a thoracotomy was made on the left side. At 10:29, during temporary hemostasis in the descending aorta, the wall was incised and a paraffined aluminum tube was introduced. The aorta was small and the tube too large for it. At 10:31 the circulation was reëstablished. The tube was fixed by two silk ligatures. On account of the distension of the aorta by the tube, the incision could not be sutured. The thoracic cavity was closed and the operation completed at 11 A. M. The animal remained normal for nine days, but on the tenth day (February 26) it died suddenly.

Autopsy.—There was no hemorrhage in the pleura. The tube, however, was occluded by a clot. The deposit of fibrin seemed to have started from a laceration of the wall at the level of the upper end of the tube. The laceration was apparently due in some measure to pressure exerted upon the wall, by the edge of the tube. The tube was corroded and the aortic wall and the surrounding connective tissue were very much thickened.

Experiment 8.—Black and white female dog, No. 992. On February 27, 1912,

at 10:06 A. M., under ether anesthesia by the Meltzer-Auer method, a left thoracotomy was made through the fourth intercostal space. The left part of the arch of the aorta was dissected and at 10:36 the aorta was clamped near the left innominate artery. Into the vessel a straight paraffined glass tube was then introduced. The tube was fixed by two silk ligatures, and at 10:38 the circulation was reëstablished and the aortic incision sutured. The pleura was also sutured around the vessel, the operation being finished at 11:12. The animal remained in excellent condition for three days, but on the morning of March 2, it was found dead in its cage.

Autopsy.—The tube was found to be occluded by a clot. This tube was straight and its sharp edges had lacerated the aortic wall. The thrombus appeared to have started in the lacerated region.

Experiment 9.—Black and white female dog, No. 1017. On March 1, 1912, at 10:41, under ether anesthesia by the Meltzer-Auer method, a left thoracotomy was made through the fourth intercostal space. This was followed by dissection of the arch of the aorta, of the first part of the innominate artery, and of the upper part of the descending aorta. At 10:58, during temporary hemostasis, the aortic wall was incised and a curved paraffined glass tube 3 mm. in length and 10 mm. in diameter was introduced into the lumen of the vessel and fixed by two silk ligatures. The circulation was reëstablished at 11 A. M. and the incision in the aorta was sutured. The thoracic cavity was then closed and the operation ended at 11:25. The dog remained in excellent condition for five days but died suddenly on March 7, 1912.

Autopsy.—The upper end of the tube was too sharp and had lacerated the wall. To this lacerated surface a clot that completely occluded the tube was adherent.

Thoracotomy, under ether anesthesia by the Meltzer-Auer method, and handling of the thoracic aorta are not dangerous. No postoperative pleuritis or other complication was observed, and during the first week following the operation, nearly all the animals remained in excellent health.

One animal (experiment 3) is living and normal at the beginning of the fourth month after the operation (April 3).[2] In this animal a glass tube of relatively small caliber with smooth edges was introduced into the descending aorta, and fixed there by a single ligature of coarse silk thread.

In two cases (experiments 1 and 4), the animals died of hemorrhage. In experiment 4 a thin-walled glass tube had been used. Eight days after the operation, the animal was apparently in excellent health, but died suddenly on this day. The pleural cavity was

[2] On May 2 the animal became suddenly paralyzed and was chloroformed. The autopsy showed that the upper edge of the tube had ulcerated the posterior part of the aortic wall, and that the tube was occluded by a fresh clot.

full of blood and the tube was found broken in the middle. The lumen was free from paraffin and fibrin. There was no ulceration of the aortic wall. In experiment 1 the tube had been fixed by two very tight ligatures which, after eleven days, cut the arterial wall, causing a sudden hemorrhage and the death of the animal. The use of a metallic tube and of ligatures properly tied would have prevented these accidents.

In experiment 6 the animal remained in excellent health for more than fifty days, but then became slightly ill and died rather suddenly, fifty-five days after the operation. Both pleural cavities were filled with a clear hemorrhagic fluid. Around the intubated portion of the aorta was a large tumor adherent to the posterior thoracic wall. This tumor was composed of granulation tissue in the center of which was found the aluminum tube. The tube was corroded and partially occluded by a deposit of fibrin. In experiment 7 an aluminum tube had also been used. The animal died rather suddenly twelve days after the operation. The tube was corroded. Its lumen was obliterated by a thrombus, and the intubated part of the aorta was very much thickened. In both cases the complications could probably have been prevented by the use of tubes made of gold instead of aluminum. In four other cases occlusion of the aorta or of the tube occurred. In three animals (experiments 2, 8, and 9) the deposit of fibrin was due to a laceration of the wall produced by the upper end of the tube. The tubes were too large and had distended the aorta. It is probable that tubes small enough to have produced no distension of the aortic wall would have caused no laceration. In the fourth animal in which an occluded aorta was found (experiment 5), a glass tube with sharp edges had been introduced into the descending aorta. This animal remained in excellent condition for fifty-eight days but then died rather suddenly. The aorta was occluded just below the lower end of the tube by a fibrinous clot that was adherent to a laceration of the wall produced by the edge of the tube. No laceration was caused by the upper end, and the lumen of the tube was entirely free from thrombus. This observation showed that blood could circulate for almost two months through a glass tube without the development of a thrombus. The laceration caused by the sharp edge seemed to be responsible for the obliteration of the vessel.

These experiments have shown that under certain conditions aortic blood can flow through a glass tube for more than three months without the occurrence of an obliterative thrombus. If the aortic wall was lacerated, a deposit of fibrin took place, and caused a partial or complete occlusion of the tube or of the vessel. The success or failure of the intubation depended upon the presence or absence of laceration of the vascular wall. It is probable that the use of a tube of proper caliber, form, and composition, for instance, a smooth edged, gold tube of relatively small caliber, would be followed by better results. It is possible also to line the tube with a vein in order to prevent more surely the occurrence of a thrombus. Further experiments will show whether complications can be completely eliminated, and whether the operation can be made safe enough to be used on human beings in case of aneurysms of the aorta.

A PHYSIOLOGICO-PATHOLOGICAL STUDY OF A CASE OF HEART-BLOCK OCCURRING IN A DOG AS A RESULT OF NATURAL CAUSES.*

By GEORGE BACHMANN, M.D.

(From the Laboratory of Physiology, Atlanta College of Physicians and Surgeons, Atlanta, Ga.)

Plates 1–7.

In the course of an investigation upon the action of strophanthus on the heart in complete auriculo-ventricular dissociation, it was the good fortune of the writer to find a dog with heart-block. This condition, which can be recognized readily by registering the movements graphically or the electrical variations of auricles and ventricles, has been studied experimentally on the mammalian heart by a number of investigators, notably by Humblet (1), Hering (2), and Erlanger (3). In accordance with the results obtained by these observers, it is generally believed that an injury to the auriculo-ventricular bundle will interfere with the free passage of the excitation wave which, in man, is normally conducted from auricle to ventricle within the narrow limits of 0.15 to 0.2 of a second.

The interference with the passage of the excitation wave varies directly with the extent of injury to the bundle.[1] This can be demonstrated easily by means of Erlanger's clamp with which it is possible to produce, by gradual compression of the bundle, all grades of interference with a-v conduction, from a prolongation of the a-v interval, to a dropping out of one ventricular contraction in 10, 9, 8, 7, 6, 5, etc., auricular contractions until only every second or third excitation wave is able to force the block and excite the ventricle to contraction (2:1 and 3:1 rhythm). Finally, a condition of complete block is obtained in which auricles and ventricles

* Received for publication, April 12, 1912.

[1] The term injury is used here in a broad sense, not necessarily implying a destruction, either partial or complete, of essential elements, but including in its meaning an impairment of functional activity.

beat independently of each other and with entirely different rhythms.

Following these physiological researches, numerous observations of auriculo-ventricular dissociation were made on man by clinicians. A fair number of these clinical cases came to autopsy and in almost all of them a lesion of some essential part of the conducting system was found. The results of clinico-pathological studies, however, are not so uniform as those yielded by physiological experiment. A perusal of the somewhat extensive literature on the subject leaves the impression that the relationship existing between lesions of the auriculo-ventricular bundle and auriculo-ventricular dissociation is still ill defined. It is obvious, therefore, that the elucidation of this problem requires further work along the lines suggested by the best of the physiological and pathological studies already made.

The unique case here reported is of especial interest because it was possible to apply in its study the direct methods of the physiological laboratory.

PHYSIOLOGICAL PART.

Technique of the Experiment.—The dog, a female weighing 7,550 grams, was in the kennel for four days previous to the experiment. There was nothing abnormal in its actions, and though not under constant observation, it was never seen in any apoplectiform seizures such as are common in cases of heart-block in man, and in dogs with an experimental lesion of the a-v bundle of some duration (Erlanger (4)). The animal was rather remarkable for its quietness, resting in a normal position the greater part of the time. No observations were made on the pulse before the experiment.

The dog received four cubic centimeters of a 2 per cent. solution of morphin sulphate hypodermically and twenty minutes later was fully anesthetized by means of ether. Both vagi were then exposed in the neck and ligatures passed under them; a small cannula filled with sodium carbonate solution was tied in a crural vein and later connected with a burette containing a solution of strophanthin (0.025 per cent.) in physiological saline. The chest was opened in the usual way, under artificial respiration; care was taken during the entire experiment to secure thorough ventilation of the lungs, as

a defective ventilation is capable of causing serious impairment of a-v conduction (5). The pericardium was slit open and the edges stitched to the sides of the opening in the chest wall. The right auricular appendage and the right ventricle were connected to tambours and their movements registered on the Hürthle kymograph. A time marker giving tenths of seconds and a signal magnet, in circuit with the primary coil of an inductorium, were placed beneath the levers of the recording tambours. All levers wrote on the same perpendicular line.

In the series of experiments to which this one belongs, a record of the heart's action was first obtained; that strength of rapidly repeated induced currents which would be sufficient to cause complete arrest of the whole heart was determined, and finally an attempt was made to crush completely the auriculo-ventricular bundle by means of Erlanger's clamp. This attempt was generally successful. In this particular instance, however, owing to a timely recognition of the abnormal action of the heart, the clamp was not applied.

On first opening the chest, the heart was observed to beat less frequently than is usually the case in dogs under morphin-ether anesthesia; there was also some arrhythmia, the beats having a tendency to occur in groups of two; the second beat was not an extrasystole. The auricles were beating so feebly that distinct pulsations could not be discerned. As stimulation of the vagus is often followed in a short time by more vigorous auricular contractions, this nerve was stimulated three times (from 1 to 5 seconds) at intervals of a few seconds. Although the current was a moderately strong one, the ventricles did not stop, neither were they appreciably slowed. The auricles, however, soon after vagus stimulation, gave contractions which were strong enough to be clearly recorded (beginning of figure 1); the degree of arrhythmia decreased also. It became then quite obvious that the low frequency noted in the ventricle was also present in the auricle, and that the contractions of the two chambers bore their normal time relation. The inference could therefore be drawn that the abnormal infrequency of the heart's action was due to overaction of the vagus.

Effect of Cutting the Right Vagus.—In order to test this hypothesis, the right vagus was cut, whereupon the auricular contrac-

tions more than doubled their former frequency (from 42.21 they rose to 99.83 per minute); at the same time they became quite regular and increased noticeably in force. The rate of the ventricles, however, remained unchanged, but their arrhythmia became a little more accentuated. Simultaneously with the increase in auricular rate there appeared, therefore, a dissociation of the action of auricles and ventricles (figure 1).

Two possibilities offer themselves in explanation of this phenomenon. The a-v dissociation might be due to an anatomical interference with the conductivity at the auriculo-ventricular junction, an interference which became manifest only upon an increased auricular rate and coincident decrease in the strength of the individual auricular impulses; or it might be due to the unopposed action of the left vagus nerve.

This second possibility is suggested by the recent observations of Cohn (6) and of Robinson and Draper (7). These observers found that in a number of instances, stimulation of the right vagus was followed by stoppage of the entire heart, while stimulation of the left vagus had but slight effect upon the auricles, although the ventricles were more or less inhibited, so that various grades of heart-block occurred. They infer, therefore, that the right vagus acts principally upon the chronotropic property of the heart muscle, while the left vagus influences principally its dromotropic property. Differences in the action of the two vagi are phenomena well known to physiologists; as far as the writer is aware, however, there has been no systematic study made regarding the percentage of cases in which the specific differences mentioned above may be found.

It is at least doubtful whether this case can be explained upon these premises; if the a-v dissociation were due to the unopposed action of a hypertonic left vagus, stimulation of the peripheral end of the right vagus with maximal induced shocks would certainly inhibit the whole heart completely, but as we shall see later, such stimulation was without effect upon the ventricles. The writer is therefore forced to the conclusion that the interference with a-v conduction was due to an anatomical block at the junctional tissues and not to hyperfunction of the unopposed left vagus. Such hyperfunction would, at any rate, be extremely rare, for, out of a fairly

large number of observations (incidental to class demonstrations) in which the right vagus alone was cut, this is the only instance in which a-v dissociation occurred. The reasons for the absence of a-v dissociation previous to section of the right vagus, in spite of the existence of an anatomical block, are given below.

The degree of block seems, on first inspection, to be slight; the tracings (figures 1, 2, and 3) show auriculo-ventricular rhythms varying from 1:1 to 2:1 and 3:1. A measurement of a number of a-v intervals gives figures ranging from 0.1 to 0.33 of a second; on the other hand, it happens sometimes that auricles and ventricles begin their contraction simultaneously.

A diagnosis of partial a-v block could be made were it not that a number of factors point to a conclusion which is, in all probability, more correct; namely, that the block is relatively complete. When the block is partial a sudden increase in auricular frequency is accompanied by ventricular stoppage which is then followed by a higher degree of block than existed before the rise in auricular rate. Such a phenomenon does not occur in absolutely complete block, but it may occur in relatively complete block.

Erlanger (8), who studied the reaction of the ventricle to increased auricular frequency in various degrees of a-v block, suggests that the rise in the rate of auricular contractions is accompanied by a diminution in the strength of the impulses transmitted to the ventricles. The sudden withholding of adequate impulses to the ventricles in partial block brings the ventricles to a standstill until they have developed their individual rhythm or until their irritability has reached such a height that they respond to a weak stimulus. In absolutely complete block, a rise in auricular frequency is not followed by stoppage of the ventricles for the reason that the ventricles have become entirely independent of the auricular impulses for their contraction, and have fully developed their own rhythmicity.

It is evident from this brief statement of Erlanger's findings that the duration of ventricular stoppage in partial block will depend on two factors, provided the frequent auricular impulses remain of uniform strength; viz., (1) the degree of irritability of the ventricular muscle, and (2) the nearness of the ventricular contractions to that rate characteristic of their inherent rhythm.

The degree of irritability of this dog's heart cannot be stated with certainty in the absence of a special inquiry into this property of the heart muscle; there is, however, no reason for believing it to be abnormal. The ventricle will be nearest the full development of its inherent rhythm when the partial block is of high grade, and particularly of that grade which Erlanger designates as relatively complete block. Whether ventricular stoppage occurs or not when the auricles are accelerated, will depend on the length of the ventricular cycle which in turn depends generally on the degree of a-v block. The longer the ventricular cycle, the nearer will be the full development of the idioventricular rhythm. The measurement of a number of ventricular cycles before section of the right vagus gives durations varying from 1.2 to 1.8 seconds; the longest cycle immediately following section of the vagus occupies 2 seconds. This cannot be regarded as a stoppage of the ventricles, as a number of cycles which occurred later and were independent of auricular impulses, had a duration of 1.8 to 2.1 seconds.

We may therefore consider the a-v block, here, to be of a very high grade though not absolutely complete. When the auricles are beating at a low rate and the excitation wave is presumably of high intensity, each auricular beat is followed by a ventricular contraction; in other words, the impulse is able to force the block; moreover, the conduction time is normal (0.1 of a second, first part of figure 1). On the other hand, when the auricles are beating at a high rate with a corresponding decrease in the intensity of the excitation wave, several auricular impulses fail to excite the ventricles to contraction. The rhythms that ensue vary considerably. There may be seen 1:1, 2:1, 3:1 rhythms and complete block, recurring very irregularly. The conduction time, when the block appears to be partial, varies from 0.1 to 0.33 of a second[2] (second part of figure 1). Owing to the fact that auricular impulses force the block irregularly, the ventricles exhibit an aperiodic form of arrhythmia.

The Effect of Stimulation of the Peripheral End of the Cut

[2] This last figure would indicate an abnormally long conduction time; it is possible that what appears under such circumstances to be a true a-v rhythm is nothing more than a coincidence.

Vagus.—Erlanger (9) has shown that the duration of ventricular stoppage which follows tetanic stimulation of the vagus with maximal stimuli decreases as the degree of a-v block increases. When the block is complete, little or no slowing of the ventricles results. This slowing occurs some little time after the cessation of vagus stimulation; viz., in five to seven seconds in Erlanger's experience.

In my experiment the peripheral end of the right vagus was stimulated with maximal tetanizing shocks. Of five observations, four were made early in the experiment, while the last one was made during the rise of ventricular frequency which followed the injection of strophanthin. In all these tests except the last, the auricles came immediately to a complete standstill when stimulation began. Direct inspection of the auricles did not reveal the presence of the least contraction in these chambers. Furthermore, their stoppage continued for some time after the cessation of stimulation. The auricles, following complete inhibition, did not return immediately to their former rate, but after the first beat, two, three, or more auricular cycles took place with durations greater than the average. The first auricular contraction, on recovery, was much higher than the average.

There can be do doubt, therefore, that the stimuli sent into the vagus were of maximal strength, and yet such stimulation was practically without effect on the ventricles. An examination of table I shows that during the stimulation no slowing is present; and after stimulation has ceased, the slowing that occurs is insignificant and is found in the second or third beat following. At this time the auricles have practically recovered. A measurement of ventricular cycles beyond those shown in the table has failed to reveal any further slowing. There is a slight difference, therefore, between these results and those of Erlanger, who found that the maximal slowing of the ventricles did not occur until six to eleven ventricular beats had taken place following the beginning of stimulation. This difference is but one of degree and may be due to the fact that the duration of stimulation of the vagus in Erlanger's experiments was much greater than in those here reported.

There can be no doubt that when the a-v block is complete, or at least of very high grade, it is impossible to produce a decided

inhibition of the ventricles by vagus stimulation (figures 2, 3, and 4, and table I).[3] A number of experiments performed on other dogs with complete a-v block, produced by crushing the a-v bundle, gave the same results. Erlanger's conclusion, therefore, that the vagus exerts little or no direct chronotropic influence on the ventricles would seem to be justifiable.

On the supposition that inhibitory impulses pass from auricles to ventricles along the same route as the a-v bundle, it does not appear from Erlanger's experiments that the structure concerned in this action is capable of regenerating, unless it be assumed that the character and intensity of the experimental lesion produced by this author precluded the possibility of regeneration. By electrical stimulation of the ventricles to a frequency higher than the rate of the independent ventricle simultaneously with vagus stimulation, Erlanger and Hirschfelder (12) have demonstrated that it is not the slow rate of the ventricles that causes them to manifest little or no inhibition.

The usual results of vagus stimulation in a-v block were likewise obtained by Erlanger (13) during the acceleration of the ventricles that followed the intravenous injection of digitalin. These findings are confirmed here by the result that followed vagus stimulation during the notable acceleration seen after the intravenous injection of strophanthin. At this time the auricles were beating at the rate of 148.88 and the ventricles at the rate of 153.41 per minute. Stimulation of the vagus brought the auricles (after one contraction following the onset of stimulation) to a complete standstill lasting 3.4 seconds. The duration of stimulation of the vagus was 1.4 seconds. In spite of this apparent increase in susceptibility of the heart muscle to vagal inhibition, no corresponding effect was observed in the ventricles. There was a disappearance of extrasystoles for a short period, but no distinct slowing (table I and figure 4).

[3] In a recent article by Fredericq (10), this statement, as well as those that follow as a corollary, is disputed. This investigator shows that complete block may be produced by appropriate pressure upon the a-v bundle without interfering with the usual inhibitory action of the vagus upon the ventricles; greater pressure abolishes the latter effect. Garrey (11) obtained similar results for the turtle's heart at the sino-auricular junction.

TABLE I.

The Effect of Stimulation of the Peripheral End of the Vagus Nerve.

Before stimulation.		During stimulation.		After stimulation.		Duration of stimulation.	Duration of auricular stoppage.
Duration of successive		Duration of successive		Duration of successive			
As cycles.	Vs cycles.	As cycles.	Vs cycles.	As cycles.	Vs cycles.		
0.5, 0.7, 0.7, 0.6, 0.6, 0.6, 0.6, 0.6	1.8, 1.7, 1.8	0	1.8	0.6, 0.8, 0.7, 0.6, 0.6, 0.7, 0.7, 0.6, 0.6, 0.6, 0.6, 0.6	1.5, 2.1, 0.6, 1.9, 1.2, 1.8	1.8	3.3
Thirteen cycles of 0.6 sec. each	1.7, 1.7, 1.7, 0.9, 1.7	0	1.8	0.7, 0.8, 0.6, 0.6, 0.6, 0.6, 0.6, 0.6, 0.6, 0.6	1.1, 2.0, 1.9, 1.8	1.2	2.8
Eleven cycles of 0.6 sec. each	1.8, 1.8, 0.8, 1.8	0	1.8	0.6, 0.7, 0.7, 0.7, 0.6, 0.6, 0.6, 0.6, 0.6, 0.6, 0.6, 0.6, 0.6	1.3, 1.3, 2.0, 0.7, 1.8	1.2	2.3
Nine cycles of 0.6 sec. each	1.9, 1.3, 1.8	0	1.9, 1.9	0.5, 0.7, 0.7, 0.6 0.6, 0.6, 0.6, 0.6, 0.6	1.2, 1.3, 1.9, 0.7, 1.8	2.6	4.1
Twelve cycles of 0.4 sec. each	0.45, 0.3, 0.45, 0.5, 0.3, 0.35, 0.35, 0.3, 0.35, 0.4, 0.3, 0.35, 0.4	0.4, 0	0.4, 0.3, 0.45, 0.3	0.5, 0.5, 0.5, 0.5, 0.5, 0.5, 0.5, 0.5, 0.4, 0.4, 0.4, 0.4	0.4, 0.4, 0.4, 0.4, 0.4, 0.4, 0.4, 0.4, 0.35, 0.5, 0.4, 0.3, 0.45, 0.4, 0.4, 0.4	1.4	3.4

The Reaction of the Heart to Strophanthin.—The pharmacodynamics of cardiac drugs, particularly of those of the digitalis series, has received a great deal of attention, both from pharmacologists and from experimental therapeutists. Thus it has been established that the action of strophanthus on the cardio-inhibitory apparatus is practically identical with that of digitalis. This fact, however, is not so generally accepted as one might suppose, and numerous clinicians adhere still to the belief that the inhibition of the heart obtained under the administration of strophanthus is due to a direct action of the drug on the cardiac muscle and not to a stimulation of the cardio-inhibitory apparatus. We have in the condition of complete a-v dissociation a simple means of demonstrating that this belief is based on erroneous premises, since, as we have seen, the ventricles are under such conditions practically free from vagal influence. On the other hand, the auricles remaining under the control of the vagus, any effect which the drug may exert upon the inhibitory apparatus will become apparent in the behavior of the auricles. It will be possible, therefore, to determine *simultaneously* any effect which the drug may have upon the cardio-inhibitory apparatus on the one hand, and upon the heart muscle on the other hand, by observing the action of the auricles in the first case, and that of the ventricles in the second case.

The preparation used was strophanthin (Merck's crystalline). A solution of the strength of 0.025 per cent. was made in physiological saline. This solution was injected from a burette into a crural vein, as explained under the technique. Each dose was one cubic centimeter and the time interval between each injection varied from four to seven minutes.

The first effect of strophanthin was to cause a decrease in the rate of the auricles and ventricles. The lowering in frequency of both auricles and ventricles is not due, however, to the same cause. The auricles are inhibited through the stimulating action of strophanthin on the cardio-inhibitory center. This fact can be determined readily by injecting by the same method a single dose of the same preparation or of the tincture of strophanthus into a dog with an intact heart. The auricles slow gradually until complete inhibition is established. This inhibition disappears on section of both

vagi, or following the injection of atropin (Gottlieb and Magnus (14), Kochmann (15), Liagre (16)). During the complete arrest of the auricles, the ventricles beat at the rate characteristic of their own rhythm.

The decrease in auricular frequency became manifest after the first injection of strophanthin; during this time the auricles beat rhythmically (figure 5). With the advent of stronger inhibition following the second injection, the auricles beat arrhythmically, the arrhythmia being occasioned by the irregular occurrence of pauses (figure 6) which became progressively longer following the third injection; the height of the auricular contractions decreased with the lowering in their frequency.

As mentioned before, the ventricular rate was likewise diminished. This slowing of the ventricles, however, cannot be due to any chronotropic influence of the vagus on these chambers since direct stimulation of the peripheral end of the cut vagus was practically without effect on the ventricular rate. There is, however, simultaneously with the decrease in ventricular frequency, a decrease in the conductivity across the a-v bundle. In other words, the vagus under the action of strophanthin, exercised a well defined negative dromotropic influence on the junctional tissues. This is shown by the fact that with the decrease in ventricular rate the ventricles became rhythmical and by the further observation that the a-v block from being relatively complete became absolutely complete. Auricles and ventricles beat with entirely independent rhythms (figure 5).

It is true that the ventricular rate fell as low as 27.49 per minute, a rate of idioventricular contraction which is abnormally low for the dog's heart; but it is necessary to take into consideration here the special circumstance that in this animal the ventricular rate was at no time very high, in fact the average ventricular rate, preceding the administration of strophanthin and exclusive of the periods during which the vagus was stimulated, was 38.32 per minute. The duration of those ventricular cycles which were apparently not disturbed by auricular impulses varied from 1.8 to 2.1 seconds; under these conditions the idioventricular rate varied from 33.33 to 28.57 per minute, figures which are practically

the same as those found under the first phase of the action of strophanthin, viz., from 32.72 to 27.49 per minute (table II). The conclusion is therefore justifiable that if strophanthus exerts any direct inhibitory action on the heart muscle, it is so slight as to be negligible.

The action of strophanthin on the cardio-inhibitory mechanism was soon overshadowed by a rise of the irritabiiity of the cardiac muscle, so that in spite of the fact that the vagus was likewise in a state of heightened irritability, as shown by the effect of faradic stimulation, the auricular and ventricular rates increased. Coincidently with the rise in the frequency of the auricular contractions the auricles became regular again, but there was no increase in the force of their beats (figure 8). Previous to the increase in ventricular rate, the conductivity across the a-v bundle improved so that, with an anatomical block which was not quite complete, the ventricles became again irregular from the occasional arrival of an adequate excitation wave from the auricle.

The rise in ventricular irritability manifested itself at first by the occurrence of an occasional extrasystole; later by the appearance in groups of a number of beats succeeding each other rapidly (figure 7). Shortly afterwards the ventricular acceleration became well established and rose progressively to a high degree; simultaneously, the height of the contractions increased. The rise in the irritability of the heart muscle occurred after the fourth injection (one milligram of strophanthin).

The auricular rate before the fifth injection was 132 per minute; that of the ventricles, 157.24 per minute: the auricular contractions were irregular and weak but quite distinct, while the ventricular contractions were regular and vigorous. Following the fifth and sixth injections, the auricular rate rose to 164.37 per minute, and afterwards declined rather rapidly to complete arrest preceded by group beating (figure 9). The auricles stopped approximately four minutes before the ventricles. If atropin be injected into the jugular vein immediately after auricular stoppage, the auricular contractions may return; shortly afterward they again stop, this time not to contract again. The auricular stoppage under excessive doses of strophanthin is therefore due primarily to a

TABLE II.

Dog VIII. Table Based on Protocols of Experiment of August 12, 1911.

Time.	Phase of experiment.	No. of Vs cycles.	Duration in $\frac{1}{10}$ sec.	Ventricular rate per min.	No. of As cycles.	Duration in $\frac{1}{10}$ sec.	Auricular rate per min.	As : Vs	Remarks.
12:03	Heart's action as found on opening chest.	28	398	42.21	28	398	42.21	1.00	Heart (As and Vs) slightly irregular.
12:05	Immediately after section of rt. vagus.	42	589	42.80	100	601	99.83	2.33	As regular; Vs slightly irregular.
12:07	Continuation	11	182	36.26	31	182	102.19	2.81	As regular; Vs slightly irregular.
	Stimulation of peripheral end of rt. vagus								See table I.
	After complete recovery	12	189	38.09	33	196	101.02	2.65	As regular; Vs slightly irregular.
	Stimulation of vagus								See table I.
	After complete recovery	37	497	44.66	99	503	118.09	2.64	As regular; Vs slightly irregular.
	Two stimulations of vagus								See table I. Interval between stimulations, 31 sec.
	After complete recovery	31	445	41.80	75	472	95.33	2.27	As regular; Vs slightly irregular.
12:11	Continuation	7	99	42.42	16	100	96.00	2.26	As regular; Vs slightly irregular.
	During first injection	37	516	43.02	84	521	96.73	2.24	0.025 per cent. strophanthin solution, 1 c.c. As regular; Vs slightly irregular.
	Immediately after	33	528	37.50	79	532	89.09	2.38	As regular; Vs slightly irregular.
	Continuation	36	660	32.72	94	664	84.93	2.59	As and Vs regular.
12:15	Continuation	55	1025	32.19	152	1038	87.86	2.72	As and Vs regular.
	During second injection	9	173	31.71	25	173	86.70	2.78	0.025 per cent. strophanthin solution, 1 c.c. As and Vs regular.
	Immediately after	31	599	31.03	89	598	89.29	2.87	As and Vs regular.
12:19	Continuation	31	650	28.61	91	662	82.47	2.88	As irregular (inhibited); Vs regular.
	During third injection	7	149	28.18	20	153	78.43	2.78	0.025 per cent. strophanthin solution, 1 c.c. As irregular (inhibited); Vs regular.
	Immediately after	32	662	29.00	89	658	81.15	2.79	As irregular (inhibited); Vs regular.
	Continuation	17	303	28.01	42	361	69.80	2.45	As irregular (inhibited); Vs regular.
12:22	Continuation	23	502	27.49	56	512	65.62	2.38	As irregular and weaker (strongly inhibited); Vs regular.
	Continuation	33	680	29.11	66	681	58.14	1.99	As irregular and weaker (strongly inhibited); 2 ventricular E.S., Vs otherwise regular.
	Continuation	13	190	41.05	17	189	53.96	1.30	As irregular and weaker (strongly inhibited); sudden acceleration of Vs.
	Continuation	26	443	35.21	46	455	60.65	1.72	As irregular and weaker (strongly inhibited); 1 ventricular E.S., Vs otherwise regular.
12:26	Continuation	9	173	31.21	25	182	82.41	2.64	As irregular and weaker (strongly inhibited); 1 ventricular E.S., Vs otherwise irregular.
	During fourth injection	11	200	33.00	26	206	70.87	2.14	0.025 per cent. strophanthin solution, 1 c.c. As the same; Vs slightly irregular.
	Immediately after	41	716	34.35	99	715	83.07	2.41	As irregular and weaker; Vs slightly irregular.
	Continuation	25	344	43.60	57	347	98.56	2.28	As regular and weak; Vs accelerated in groups of three beats.

									1 c.c. As the same; Vs slightly irregular.
	Immediately after	41	716	34.35	99	715	83.07	2.41	As irregular and weaker; Vs slightly irregular.
	Continuation	25	344	43.60	57	347	98.56	2.28	As regular and weak; Vs accelerated in groups of three beats.
12:30	Continuation	17	140	72.85	26	141	110.63	1.51	As regular and weak; Vs accelerated irregularly.
	Continuation	38	221	103.16	42	226	111.50	1.08	As regular and weak; Vs accelerated regularly.
	Continuation	48	203	141.37	42	204	123.52	0.87	Heart's action the same.
	Continuation	42	182	138.46	37	182	121.98	0.88	Heart's action the same.
	Continuation	158	637	148.82	136	639	127.69	0.87	Heart's action the same.
12:34	Continuation	38	145	157.24	33	150	132.00	0.84	Heart's action the same.
	During fifth injection	38	145	157.24	33	149	132.88	0.84	0.025 per cent. strophanthin solution, 1 c.c. As regular and weak; Vs regular.
	Immediately after	90	345	156.52	79	347	136.59	0.87	As regular and weak; Vs regular.
	Continuation	101	395	153.41	98	395	148.86	0.97	As regular and weak; Vs irregular (numerous ventricular E.S.).
	Stimulation of vagus								See table I.
	After complete recovery	177	645	164.65	179	654	164.37	0.99	As regular and weak; Vs irregular (numerous ventricular E.S.).
12:38	Continuation	62	208	178.84	55	212	155.66	0.87	As regular and weak; Vs regular.
	During sixth injection	53	177	179.54	43	177	145.76	0.81	0.025 per cent. strophanthin solution, 1 c.c. As regular and weak; Vs slightly irregular (a few ventricular E.S.).
	Immediately after	254	802	190.02	168	802	125.68	0.66	Group beating of As; Vs regular.
12:41	Continuation	131	439	179.04	51	439	69.70	0.38	Group beating of As; Vs regular.
12:43	Continuation	113	373	181.76	0		0		As stopped; Vs regular.
	Continuation	36	124	174.19	0		0		As stopped; Vs regular.
	Continuation								Fibrillation of Vs followed by arrest in diastole (12:44).

return of vagal inhibition, and secondarily to a loss of irritability through the poisonous action of the drug on the cardiac muscle.

A careful analysis of this and other similar experiments leads to the inference that the increase in the tone of the cardio-inhibitory apparatus under the influence of strophanthus lasts practically the entire time that the auricle is beating. The increased activity of the cardio-inhibitory mechanism is unable to make itself felt during the notable rise of irritability of the heart muscle which follows moderately large doses of the drug. As soon as the irritability declines sufficiently, however, vagal inhibition is manifested by complete stoppage of the auricles. During this stage, lasting but a short time, the intravenous injection of atropin is followed by a return of the auricular beats. The auricular irritability continues to decline rapidly until all auricular contractions cease permanently. Curiously enough, while the auricles are the more irritable parts of the mammalian heart and the right auricle is the ultimum moriens under normal conditions, these chambers are nevertheless, the first to succumb to the toxic action of strophanthus. The ventricular contractions following the fifth and sixth injections became irregular owing to the occurrence of extrasystoles; the ventricular frequency rose to 190.02 per minute to decline in a short time to 174.19 per minute. From this relatively high frequency the ventricles passed suddenly into fibrillary contractions and stopped in the diastolic position.

The quantity of strophanthin necessary to bring about the death of the heart was therefore 1.5 milligrams. As the animal weighed 7,550 grams, the lethal dose was 0.1986 of a milligram per kilo of body weight. This dose is smaller than the one given by Étienne (17), who determined that the minimal fatal dose of strophanthin (Merck) in the dog is 0.25 of a milligram per kilo when administered intravenously. His animals were neither anesthetized nor operated upon, circumstances which doubtless played an important part in their resistance to the drug.

PATHOLOGICAL PART.

The Gross Appearance of the Heart.—Immediately after the complete arrest of the heart, this organ was removed, its chambers

were opened, and the clots that had formed in the right auricle washed out. The following is taken from the protocols.

Heart.—Color and size normal. Weight 60.5 grams.

Right Side of Heart.—The posterior (septal) leaflet of the tricuspid valve is considerably thickened at its free edge; this thickening is smooth, firm, elastic, and semitransparent (resembling myxomatous tissue). Immediately above the attached portion of this valve and five millimeters from the opening of the coronary sinus, the auricular septal musculature shows an oval patch which is grayish in color and is traversed in all directions by fine dark red lines resembling enlarged capillaries. This patch measures three by five millimeters and its long axis runs parallel with the auriculo-ventricular margin. The pars membranacea septi is immediately in front of this patch (figure 10).

Left Side of Heart.—The same semitransparent gelatinous thickening seen at the edge of the septal tricuspid leaflet is found also in the angle between the posterior (non-coronary) leaflet and the right anterior leaflet of the aorta; it therefore overlies the pars membranacea septi, forming there a distinct nodule. The same thickening spreads along the entire length of the attachment of the posterior aortic leaflet as well as along the right (or posterior) attached margin of the right anterior leaflet (figure 11). The rest of the heart shows nothing noteworthy.

The vagi throughout their course in the neck showed no abnormality. The medulla was likewise normal in appearance.

The proportion of heart weight to body weight is 0.00801. The average proportional weight of the normal dog's heart according to Stewart (18) is 0.00721, and in the case of a female in his series with a body weight of 7,700 grams the proportional weight was 0.00746. We may therefore conclude that little or no hypertrophy was present.

Microscopic Examination of the Septum.—The entire heart was placed in 10 per cent. formalin for fifteen days. A block of tissue was then cut from the septum as follows: the interauricular septum was cut parallel with the auriculo-ventricular junction ten millimeters above it; another cut parallel with this one was made through the interventricular septum twenty millimeters below the auriculo-

ventricular junction. These two cuts were joined anteriorly by one at right angles with them and five millimeters in front of the pars membranacea septi, and posteriorly by another cut parallel to the one just mentioned and three millimeters posterior to the opening of the coronary sinus (figure 12).

This block of tissue was dehydrated in ascending strengths of alcohol and, after thorough infiltration, was embedded in celloidin. The block was oriented in such a manner that the sections were cut in a frontal plane, so that each section included a part of the interauricular septum and adjoining structures (*e. g.,* the wall of the aorta), the auriculo-ventricular junctional tissues and the interventricular septum. The sections began anteriorly to the pars membranacea septi and were continued backward well beyond the opening of the coronary sinus.

The sections were cut twenty-five microns thick and were mounted serially. At first every fifth one was mounted, but later, as those parts of the tissue which are of interest were reached, three out of every five were selected, so that from among 900 sections obtained from the entire block, 480 were mounted. The sections were stained with Mayer's acid hemalum and Van Gieson's stain.

Before any part of the a-v bundle appeared, portions of the right limb and, later, of the left limb were found on the corresponding sides of the ventricular septum. The muscle fibers of both limbs were normal and the neighboring tissues presented nothing unusual. The succeeding sections showed a gradual rise in the position of the limbs of the bundle along the sides of the ventricular septum, so that ultimately they could be seen on each side of the lower border of the pars membranacea septi. Here, there appeared under the endocardium a tissue occurring in spherical or ovoid masses and consisting of ill defined fibers arranged more or less concentrically, giving to these masses the appearance of whorls. The fibers take a faint yellow stain except at the periphery of the mass, where in many sections they take a pink color. The nuclei are spindle-shaped. The same tissue can be seen at the free end of the septal tricuspid leaflet and at the base of the aortic leaflets in situations corresponding to those of the semitransparent swellings described in the gross appearance of the heart.

This tissue is, in all likelihood, newly developed connective tissue. The concentric arrangement of its fibers suggests that it is developed from the walls of the blood-vessels and that it might be elastic connective tissue. A few sections stained with Weigert's elastic stain, however, gave negative results.

This tissue is seen in all succeeding sections. It invades now the right, now the left side of the membranous septum; it comes in contact in several places with the fibers of the bundle, which then exhibit evidences of pressure, but nowhere does it interrupt the main body of the bundle. A few foci of round cell infiltration are seen at various points on the surface of the bundle; these, however, are not numerous nor conspicuous.

There is a large amount of adipose tissue above the entire course of the bundle (figure 16). The extensive distribution of this adipose tissue corresponds to the area covered by the oval, grayish patch seen in the gross specimen above the attached margin of the septal tricuspid leaflet. A comparison of similar sections obtained from a normal dog's heart shows that the adipose tissue has replaced a great part of the bundle fibers and of the ordinary auricular musculature. In some places the fibers of the bundle are separated by the adipose tissue into groups of various sizes (figures 13 and 15). The muscular connection between auricle and bundle (at the node of Tawara) takes place by a relatively narrow strand only, and but a short distance from the opening of the coronary sinus.

The anatomical pathway for the propagation of the excitation wave from auricle to ventricles was not completely interrupted at any point of its course. Indeed, it had, for a short distance at least, an almost normal appearance (figure 14). Along the greater part of the course of the bundle considerable pressure was evidently exerted upon its elements by the numerous whorls of new connective tissue already described. While such pressure led to structural alterations of the muscle fibers in but a few places, it is quite possible that this was the cause of the interference with the function of the conducting system exhibited in the action of the heart. A further impediment to the free action of this system was found in the relatively narrow connection between the auricular muscle and the node, as well as in the attenuation of the main bundle observed along its course.

Review of the Pathology of Heart-Block.—Most of the cases of heart-block found in the literature have been cases exhibiting the Adams-Stokes syndrome. Such cases were described by Morgagni (19) in 1761 and by Spens (20) in 1792 long before the contributions of Adams (21) in 1827 and of Stokes (22) in 1846 appeared. The number of cases published before the description of the auriculo-ventricular bundle by His (23) and others, and the subsequent study of its function, is relatively small. But even before a knowledge was gained of the part played by heart-block in the causation of the Adams-Stokes syndrome, the condition of the heart had been studied at autopsy, and Stokes, in particular, sought to establish a definite relationship between affections of the myocardium and the syndrome with which his name is associated.

A statistical study of the pathology of heart-block falls naturally into two distinct periods; viz., a period preceding the general recognition of heart-block, and one following it. There is, it is true, a period of transition during which the influence of certain opinions regarding the underlying pathology of the Adams-Stokes syndrome continued to be felt while our present conception of it was gradually spreading. This transitional period is naturally difficult to delimit and, moreover, were it possible to do this it would serve no useful purpose. For this reason, the year of His's communication (1899) has been chosen arbitrarily as the date dividing the old period from the new.

The old period is of interest chiefly from an historical point of view. From this period I have been able to collect but fifteen cases that contain more or less definite information concerning the heart (table III). Even in these old cases it is possible to pick out nine with lesions occupying such positions as to constitute presumptive evidence of implication of the auriculo-ventricular bundle.[4]

The number of cases studied clinically and post mortem since 1899 testifies to the interest of the medical profession in this curious malady. I have collected sixty-three of these cases.

The object of collecting these cases was to determine to what extent the clear cut results of physiological experimentation were corroborated by disease of the auriculo-ventricular conducting sys-

[4] These cases are indicated in the table by an asterisk.

TABLE III.

Year.	Author.	Pathologic condition.	
		Gross.	Microscopic.
1838	*Gibson, J. R. (24)	Atheroma of aorta and coronary arteries. Calcification of mitral leaflet.	Not given.
1842	*Peacock (25)	Circumscribed aneurysm at base of interventricular septum.	Not given.
1849	Cain (26)...	Hydropericardial effusion. Hypertrophy of heart. Myocardium softened.	Not given.
1856	*Goddard Rogers (27) (two cases)	Indurated areas in septum. (No details.)	Not given.
1875	Cornil (28) .	Fatty degeneration of heart. Atheroma of aorta. Valves normal.	Not given.
1879	Blondeau (29) 1.	Fatty degeneration of the heart. Arteriosclerosis.	Not given.
	Blondeau. 2.	Calcareous vegetations on aortic valves. Slight fatty degeneration of heart.	Not given.
	*Blondeau. 3.	Hypertrophy of heart. Atheroma of mitral and aortic valves. Milky patches on endocardium.	Not given.
1879	*Robinson (30) (Meigs's case.)	Gumma of septum the size of a walnut extending from tricuspid leaflet into right auricle and into left ventricle below aortic opening.	Tissue unsatisfactory for microscopic examination.
1885	Gibbings (31)	Heart and coronary arteries normal.	Not given.
1887	Frey (32) ...	Fatty degeneration of heart. Coronary arteries calcareous.	Not given.
1892	*Sendler (33)	Heart enlarged and fatty. Tumor (fibroma) the size of a walnut and of cartilaginous hardness 1 cm. below opening of pulmonary artery.	Nothing of special interest.
1893	*Dumas (34). (Devic's case.)	Hypertrophy of heart. Lesion, the size of a large hazelnut, in the posterosuperior portion of interventricular septum.	Loosely woven, newly formed connective tissue. A few hemorrhagic spots. Islets of muscle fibers more or less degenerated and surrounded by the new connective tissue.
1895	*Rendu (35).	Gumma of interventricular septum extending from base of aortic cusps to anterior papillary muscles and implicating large mitral leaflet. The tumor was surrounded by a zone of interstitial fibrosis.	Not given.

tem. This statistical investigation has been somewhat disappointing owing to the incompleteness in many instances of the data necessary for such comparison. An analysis of table IV shows, for instance, that in sixteen cases in which a lesion in the path of the a-v bundle was demonstrable macroscopically, no microscopic examination of the septum was undertaken. In these cases the de-

TABLE IV.

Year.	Author.	Degree of block.	Pathologic condition.	
			Gross.	Microscopic.
1903	Starck (36).....	Not given.....	Hypertrophy of heart. Valves normal. Under mitral valve on posterior wall of left ventricle is a whitish area, in part indurated.	Not given.
1903	Bassoe (37).....	Not given.....	Upper part of ventricular septum is very thin, white, fibrous, and transparent.	Myocardium shows thickening of vessel walls and round cell infiltration.
1904	Dufour (38).....	Not given.....	Hypertrophy of heart. Sclerosis of mitral valve. At base of small mitral leaflet and in myocardium is a calcareous tumor in a fibrous capsule.	Not given.
1904	Handford (39).....	Not given.....	Thickened mass in upper part of anterior wall of right ventricle implicating auriculo-ventricular septum. Bulging of endocardium on interventricular septum below the pulmonary valve.	Gummata with necrotic and caseous centers. Heart muscle somewhat fibrous.
1905	Debove (40).....	Not given.....	Hypertrophy and dilatation of the right side of heart. Atheroma of basilar artery.	Not given.
1905	Medea (41)			
	Case 2.....	Not given.....	Heart somewhat fatty. Atheroma of aorta. Cerebral edema.	Not given.
	Case 3.....	Not given.....	Myocardium fatty. Circle of Willis atheromatous. Hyperemia of medulla with hemorrhagic spots. Kidneys congested.	Not given.
1905	Stengel (42).....	Complete.....	Fibrocalcareous mass under the endocardium on a level with the insertion of the anterior mitral leaflet.	Not given.
1906	Chapman (43)..... (Keith and Miller (44).)	Not given.....	Interauricular septum and part of interventricular septum are transformed into fibrous tissue. Fibrocalcareous mediastinitis with complete obliteration of superior vena cava.	Beginning and more than half of body of bundle of His are destroyed.
1906	Keith and Flack (45) (Grünbaum's case)	Not given.....		Bundle implicated in gumma of septum (no details).
	(Mackenzie's 2 cases)	Not given.....		Marked fibrosis of bundle in both cases (no details).
1906	Hay and Moore (46)	Partial, and later complete.	Hypertrophy and dilatation of heart. Coronary arteries atheromatous, the right almost closed.	Atheroma of branch of right coronary which supplies bundle. Central fibrous body slightly atheromatous. Recent inflammatory focus where bundle perforates central fibrous body. The bundle is only partially implicated in this process.
1906	Gibson, G. A. (47)..	Partial.....	Obliteration of pericardial sac. Dilatation and hypertrophy of heart. No valvular lesion.	Fibrosis and cellular infiltration of bundle of His.
1906	Barr (48).....	Complete.....	Heart dilated and hypertrophied. Patchy fibrosis of aortic cusps and mitral valve toward their base, most marked at junction of mitral and right septal cusp.	Arteries near bundle show irregular patches of thickening. More than half of bundle is reduced to a fibrous condition. Terminal fibers of limbs (subendocardial fibers) show thickening of myolemma (Keith).
1906	Aschoff (49)..... (Hering's cases)	(1) Not given.....	Not given.....	Severe sclerosis of small arteries of Tawara's node and of principal bundle.

			mitral and right septal cusp.	limbs (subendocardial fibers) show thickening of myolemma (Keith).
1906	Aschoff (49)..... (Hering's cases)	(1) Not given.....	Not given.....	Severe sclerosis of small arteries of Tawara's node and of principal bundle.
		(2) Not given.....	Not given.....	Marked fatty infiltration at same place. Fatty infiltration of whole heart. No destruction of muscle fibers of conducting system.
1906	Brouardel and Villaret (50) (Case 1).....	Not given.....	Myocarditis. Atheroma of aorta. Sclerosis of coronary and cerebral arteries.	Not given.
	(Case 3).....	Not given.....	Fibrous myocarditis. Atheroma of coronary arteries.	Not given.
	(Case 6).....	Not given.....	Hypertrophy of left ventricle and interventricular septum.	Not given.
1906	Schmoll (51)..... (Case 2)	Complete.....	Hypertrophy of heart; otherwise nothing noteworthy.	Interstitial fibrosis of bundle with atrophy of its muscle cells (Ophüls).
1907	Fahr (52)..... (Case 1 of Deneke)	Complete(?).....	Numerous small foci of sclerosis disseminated in musculature, particularly in that of left ventricle.	A-v bundle normal in appearance (was not followed to its termination).
1907	Fahr..... (Case 2 of Deneke)	Not given.....	Indurated area at place where bundle usually divides into two limbs.	Origin of left limb entirely replaced by connective tissue; right limb larger and in good condition at its beginning but much altered where it fuses with ventricular musculature.
1907	Fahr..... (Luce's case)	Not given.....	Aneurysm of sinus of Valsalva of right anterior aortic cusp projecting into both ventricles. Tumor in upper half of ventricular septum. Partial parenchymatous degeneration of trunk of vagus.	Gumma which has destroyed fibers of bundle of His from place where it passes out of annulus fibrosus onward.
1907	Oddo and Sauvan (53)	Not given (probably complete).	Infarct the size of a pea at place corresponding to beginning of bundle of His.	
1907	Vaquez and Esmein (54)	Complete.....	Sclerotic patch on posterior part of interauricular septum on right side.	Sclerogummatous lesion of bundle of His, with complete interruption at pars membranacea septi. Fatty degeneration of remaining fibers.
1907	Störck (55).....	Not given	Gumma of bundle. No details.	
1907	Butler (56).....	Complete.....	Hypertrophied and dilated heart.	Fatty infiltration and degeneration of bundle of His with atrophy to $\frac{1}{5}$ its normal size.
1907	Ashton, Norris, and Lavenson (57)...	Complete.....	Gummatous infiltration of entire interventricular septum except its posterior portion, as seen from left side. Three nodules found on right side, two of which are below the middle and posterior tricuspid leaflets.	Round cell and spindle cell infiltration of bundle of His. Complete interruption of bundle by necrotic gummatous tissue.
1908	Jellinek, Cooper, and Ophüls (58).....	Complete.....	Anemic necrosis of ventricular septum in region of pars membranacea septi of a patient that had gonorrheal septicemia.	Artery in necrosed tissue was found filled with thrombi consisting of granular material and fragments of leucocytes. No explicit mention of bundle.
1908	Jagic (59).....	Not given.....	Numerous gummata of various sizes in upper part of interventricular septum.	Not given.
1908	Turrell and Gibson, A. G. (60).....	Not given (complete?)	Hypertrophy of heart. Sclerosis and patchy calcification of coronary arteries.	Fibrosis in region of central fibrous body spreading toward place occupied by bundle. A

				few isolated strands of muscle are all that remain of bundle in fibrous body.
1908	Gerhardt (61)..... (Case 3)	Partial mostly. Later, normal conduction.	Moderate synechia of pericardial sac. Small endocardial vegetation on mitral, aortic, and tricuspid valves. Heart muscle slightly cloudy.	Cellular infiltration of bundle where it passes through annulus fibrosus; especially marked in neighborhood of vessels whose lumen is narrowed by thickening of intima. Ventricular and auricular muscle normal.
1908	Gibson, A. G. (62)..	Complete.....	Heart hypertrophied. Increase in subepicardial fat.	Extensive fatty infiltration of bundle at place where it enters central fibrous body. Notable fatty infiltration of auricular muscle.
1908	Karcher and Schaffner (63).....	Not given; probably complete.	Numerous small indurated areas and thickening of endocardium.	Bundle of His contains an excess of connective tissue causing it to be much reduced in size, in certain sections more than half being destroyed.
1908	Vickery (64).....	Not given (complete?).	A fibrocalcareous mass extending from insertion of anterior mitral leaflet to base of anterior cusp of aortic valve. This mass is beneath the endocardium and penetrates into the myocardium.	Extensive fibrous and fibrocalcareous change throughout parts mentioned. No special description of condition of bundle of His.
1908	Beck and Stokes... (65)	Partial.....	Calcareous nodule below mesial aortic cusp running backward along upper part of interventricular septum to attachment of anterior mitral leaflet. A portion of the mass passed to junction of muscular with membranous part of septum.	Calcareous infiltration of auriculo-ventricular junction producing pressure on bundle of His resulting in extensive fibrous myocarditis with atrophy of muscle fibers.
1908	James (66).....	Complete.....	Large ulcer on interventricular septum (left side) beneath aortic valve. Mitral leaflets thickened.	Not given.
1908	Beeson (67).....	Not given, but probably complete.	Extreme fatty degeneration of heart with calcareous deposits. One large deposit found in tissue of auriculo-ventricular septum where bundle passes.	Not given.
1908	Heinecke, Müller, and Hösslin (68)			
	Case 1.....	Partial.....	Heart much enlarged. Endocardium of both ventricles overlying septum is white and opaque. Septum hard and inelastic. Valves normal.	Fibrosis and gummatous foci in interventricular septum. Round cell infiltration of bundle of His; no break in continuity. Vagi normal.
	Case 2.....	Partial; complete later.	Heart dilated. Valves normal except for a thickening of aortic leaflets at their point of attachment.	Place where bundle should be found is occupied by fibrous tissue strewn with calcareous deposits and small blood extravasations. No trace of bundle could be found. Vagi normal.
1909	Bramwell (69).....	Complete.....	Calcareous deposits in aortic and mitral valves (anterior leaflet of mitral valve markedly calcareous). Dissection of bundle shows apparently a fibrous degeneration of bundle. A calcareous nodule at right end of anterior mitral leaflet penetrates into septum and nearly obliterates bundle towards its auricular extremity.	Not given.
1909	Handwerck (70)....	Not given (partial?).	Interauricular septum and upper part of interventricular septum are invaded by an irregular, hard, dull red tumor.	Examination of small part of tumor tissue showed it to be a gumma. A-v bundle was not examined.
1909	Nagayo (71)	Complete.....	Calcareous mass (size of a bean) in upper posterior portion of left ventricle, in myocardium,	Old calcareous focus surrounded by connective tissue (calcareous gumma) which caused pressure

1909	Nagaya (71)	Complete	Calcareous mass (size of a bean) in upper posterior portion of left ventricle, in myocardium, and under posterior aortic cusp.	Old calcareous focus surrounded by connective tissue (calcareous gumma) which caused pressure atrophy of bundle and its complete interruption in auricular septum.
1909	Gibson, G. A., and Ritchie (72)	Partial; complete subsequently.	Hypertrophy and dilatation of heart. Segments of mitral valve thickened and calcareous; base of aortic cusps notably affected.	Dense fibrous tissue spreading from posterior aortic cusp into a-v node and first part of bundle, largely replacing normal tissue; areas of calcification present also. Fatty infiltration of tissues at junction of superior vena cava and right auricle.
1909	Souques and Chéné (73)	Partial	Fibrocalcareous lesion of interauriculo-ventricular part of septum and of upper part of interventricular septum, immediately underneath line of attachment of posterior segment of large mitral leaflet.	Not given.
1910	Magnus-Alsleben (74)	Alternating normal a-v sequence and complete a-v dissociation.	Thickening of mitral and tricuspid valves. Endocardium of left ventricle shows fibrous white line in neighborhood of a-v bundle.	Disseminated waxy parenchymatous degeneration of portions of individual fibers of a-v bundle.
1910	Bishop (75)	Complete	Calcareous nodule in path of the a-v bundle. Photograph shows nodule on left side of interventricular septum beneath aortic valve.	Not given.
1910	Krumbhaar (76)	Complete	Heart hypertrophied. Thin, opaque yellow plaques at base of mitral leaflets, and under posterior aortic cusp. Bases of aortic cusps are calcareous. Calcification of coronary arteries.	Slight increase in connective tissue at different points in bundle. Musculature of node, bundle, and greater part of limbs in good condition. Slight fibrosis of sino-auricular node.
1910	Barié and Cléret (77)	Not given (partial?).	Aortic cusps considerably thickened and very hard. Sclerosis extends to point of attachment of large mitral leaflet on the interventricular septum. Extensive sclerosis of mitral valve. Chronic endocarditis of region of interauriculo-ventricular septum.	Large bands of connective tissue arising from subendocardial sclerosis penetrate into bundle and isolate and compress its fibers.
1910	Fleming and Kennedy (78)	Complete	Heart dilated and pale. Valves normal.	Inflammatory foci in node and first part of bundle (round cell infiltration and congested capillaries). Large sized inflammatory focus about middle of bundle causing much damage to fibers. Also inflammatory foci in auricle at edge of node. Vagi normal.
1910	Pribram and Kahn (79)	Normal heart action, partial and complete block recurring periodically.	White, indurated spots on both sides of septum. A few wart-like vegetations on aortic cusps.	Hyaline degeneration of artery of fibrous septum. Slight sclerosis of a-v node; muscle, however, well preserved. Marked fatty infiltration of node and body of a-v bundle; lymphocytic infiltration of latter for a short distance. Muscle fibers of body and limbs of bundle in fair condition. Numerous areas of fibrosis near base of papillary muscles of left ventricle. Vagi near heart normal (W. Koch).
1910	Beck and Stokes (81)	Not given (partial?).	Heart enlarged. On section, fine yellowish streaks are seen in the interventricular septum. Valves normal.	Body of bundle contains numerous hemorrhages; its two limbs are implicated in a moderate purulent infiltration found in interventricular septum also.
1911	Armstrong and Mönckeberg (82)	Complete	Nothing noteworthy.	Lymphangioendothelioma of posterior portion of atrioventricular node and of adjacent auricular

1911	Conn, Holmes, and Lewis (83)	Heart's action usually normal. Frequent recurrences of partial or complete block.	Atheroma of coronary arteries. A strand of dense connective tissue passed from aortic leaflet of mitral valve to lower part of pars membranacea septi.	Moderate fatty infiltration of Tawara's node and of body of bundle. Increase of connective tissue within bundle. Large blood sinuses reduced number of muscle fibers considerably. Almost complete isolation of left branch from body of the bundle by connective tissue, only a few fibers remaining. Right branch almost intact.
1911	Monrad-Krohn (84)	Not given	Not given	Main part of bundle destroyed and large calcareous mass found in its place.
	(2 cases)	Not given	Not given	Main portion of bundle reduced for a short distance to a small number of atrophied fibers. Sclerosis and calcareous deposits.
		Not given	Not given	No pathological change, but limbs of bundle were not followed to termination.
1911	Mollard, Dumas, and Rebattu (85)	Partial	Hypertrophy of heart. No macroscopic lesions. Dense connective tissue mass between pulmonary artery and aorta binding these vessels intimately. Left vagus in great part buried in connective tissue mass.	Auriculo-ventricular bundle normal. Condition of nerve fibers of cardiac plexus not stated.
1912	Butterfield (86)	Partial	Heart soft and pale. Vegetations on mitral, aortic, and tricuspid valves.	Dense infiltration of a-v node by lymphocytes, polymorphonuclear leucocytes, and large mononuclear cells. Lymphocytic perivascular infiltration in node, bundle, and left branch. Right branch normal. Giant cells in fibrous sheath of left branch.
1912	Price and Mackenzie (87)	Complete (?); auricles fibrillating.	Nothing noteworthy	Extreme parenchymatous degeneration and cellular infiltration of heart muscle, in ventricular myocardium especially. Muscle fibers of a-v bundle and sino-auricular node normal. Slight vacuolization of fibers of a-v bundle.

struction, either partial or complete, of the a-v conducting system or of any part of it, must remain a matter of conjecture. On the other hand, of forty-one cases in which the septum was examined microscopically, seventeen are without definite information regarding the degree of auriculo-ventricular block existing during life. Thus, out of what seems at first to be ample material, there remain but twenty-four cases which can be used for the matter under consideration. These cases show that a complete transverse lesion of the main body of the conducting system (node and bundle proper) is invariably followed by complete heart-block.

The only exception to this rule is the well known case of Heineke, Müller, and Hösslin (68), in which, despite the complete replacement of the a-v bundle by fibrous tissue, the block was partial; during the last weeks of the patient's life the block, however, became apparently complete. Whether the complete destruction of the bundle took place during the last six weeks of the patient's life can only be surmised. The authors have suggested that a slow destruction of the bundle may be accompanied by the formation of other muscular pathways for the conduction of the excitation process. Or it may be that preformed muscular pathways under such circumstances assume this new function. The smooth muscle of the subendocardium and of the blood-vessels have been mentioned in this connection by Monrad-Krohn (84).

A second group of cases includes lesions varying widely in their severity. There seems to be in this group no definite relationship between the extent of the anatomical change and the character of the heart's action. The a-v block may be partial or complete, or a partial block may become complete; again, a partial block may disappear, the heart's action becoming normal. Finally there have been reported, within recent years, cases that exhibit in periodical recurrence normal a-v sequence, partial block, and complete block. Two such cases have come to autopsy; one was reported by Pribram and Kahn[5] (79) and the other by Cohn, Holmes, and Lewis (83) (table IV).

A third group, also of recent origin and consisting at present of five observations, concerns cases of auriculo-ventricular dissociation

[5] Histological examination by Koch (80).

in which alterations in the bundle were absent or insignificant. In the first of these the block was probably complete (Fahr[6] (52)); in the second the block was complete (Krumbhaar (76)); in the third the degree of a-v dissociation is not given (Monrad-Krohn (84)); in the fourth, the block was partial (Mollard, Dumas, and Rebattu (85)); while in the fifth, the block was probably complete (Price and Mackenzie (87)).

It is unfortunate that, in none of these cases, apparently were the limbs of the bundle followed to their termination; viz., the points of fusion of the Purkinje fibers with the ventricular muscle proper. This is a matter of importance, and one which was correctly estimated by Fahr and by Monrad-Krohn when they refused to attach any great value to their own similar observations.

Evidently the number of well studied cases is insufficient to permit of any positive conclusion. The more general application of graphic methods in clinical investigations promises to furnish, in the near future, more accurate information upon the character of the heart's action, and in a greater percentage of cases than has been obtained heretofore. The post-mortem examination of the heart must be just as thorough as its ante-mortem study; it is particularly important that the whole conducting system shall be examined. An examination of the node, main bundle, and the beginning of the latter's limbs, is not sufficient; it should include besides the parts mentioned the junction of the auricular fibers with the node and the junction of the Purkinje branches with the ventricular muscle. Two cases point to the wisdom of this course; one is that of Armstrong and Mönckeberg (82) in which the lesion destroyed the connecting fibers between auricle and node, and the other, not so well defined, is that of Koch (80) (Pribram and Kahn (79)) in which a diffuse subendocardial induration seemed to have interrupted the branches of the left limb, especially at the base of the papillary muscles.

SUMMARY.

What is believed to be the first known case of heart-block arising

[6] This is the first case of Deneke, in which Fahr reported negative findings. Subsequently Fahr rejected as unjustified his first conclusion, that permanent heart-block could occur without lesion of the conducting system.

in a dog as a result of an ingenerate pathological lesion is here reported.

The auriculo-ventricular dissociation was of that degree known as relatively complete block and became apparent on section of the right vagus nerve.

Stimulation of the peripheral end of the cut vagus failed to inhibit the ventricles, although complete inhibition of the auricles occurred. The same results were obtained during the ventricular acceleration produced by strophanthin, so that the failure of the vagus to inhibit the ventricles is not due to the latter's infrequent action, but more probably to a normal lack of direct chronotropic influence upon the ventricular muscle. These findings are similar to those obtained by Erlanger in experimental heart-block.

To small repeated doses of strophanthin injected intravenously the heart reacted as follows: (*a*) irregular slowing of the auricles and conversion of the relatively complete into an absolutely complete a-v block; (*b*) a rise in the irritability of the cardiac muscle manifested by a rapidly progressing auricular and ventricular frequency, the ventricular frequency surpassing ultimately the auricular frequency; (*c*) complete arrest of the auricles, the ventricles continuing at their high rate; (*d*) sudden fibrillation of the ventricles and shortly afterwards arrest in diastole.

There were found post mortem myxomatous-like thickenings at the free edge of the septal tricuspid leaflet and at the attached margin of the posterior aortic leaflet and along part of the right anterior aortic leaflet. There was also a grayish patch on the right side of the auricular septum above the auriculo-ventricular junction.

The thickenings at the edge of the valves consisted of dense, circumscribed masses of what appeared to be new connective tissue. The same tissue was found pressing against the bundle along the greater part of the latter's course. There was considerable fatty infiltration of the auricular musculature immediately above the bundle and, to a slight extent, of the bundle itself. The fibers of communication between the auricular muscle and the node of Tawara were relatively few as compared with those of the normal heart.

A review of the pathology of heart-block is appended, showing

the present status of the question concerning the relationship existing between disease of the a-v conducting system and the various grades of heart-block.

BIBLIOGRAPHY.

1. Humblet, M., *Arch. internat. de physiol.*, 1904, i, 278; 1905–06, iii, 330.
2. Hering, H. E., *Arch. f. d. ges. Physiol.*, 1905, cvii, 97; 1905, cviii, 267.
3. Erlanger, J., *Jour. Exper. Med.*, 1905, vii, 676; 1906, viii, 8.
4. Erlanger, J., and Blackman, J. R., *Heart*, 1909–10, i, 177.
5. Lewis, T., and Oppenheimer, B. S., *Quart. Jour. Med.*, 1911, iv, 145.
6. Cohn, A. E., *Jour. Exper. Med.*, 1912, xv, 49.
7. Robinson, G. C., and Draper, G., *Jour. Exper. Med.*, 1911, xiv, 217; 1912, xv, 14.
8. Erlanger, J., *Am. Jour. Physiol.*, 1906, xvi, 160.
9. Erlanger, J., *Arch. f. d. ges. Physiol.*, 1909, cxxvii, 77.
10. Fredericq, L., *Arch. internat. de physiol.*, 1912, xi, 405.
11. Garrey, W., quoted by Fredericq, *loc. cit.*
12. Erlanger, J., and Hirschfelder, A. D., *Am. Jour. Physiol.*, 1905–06, xv, 153.
13. Erlanger, J., *Arch. f. d. ges. Physiol., loc. cit.*
14. Gottlieb and Magnus, cited by Schedel, H., *Arb. a. d. pharm. Inst. d. Univ. Berlin*, 1904, ii, 88.
15. Kochmann, M., *Arch. internat. de pharmacod.*, 1906, xvi, 221.
16. Liagre, C., *Jour. de physiol. et de path. gén.*, 1906, viii, 988.
17. Étienne, G., *Arch. de méd. expér. et d'anat. path.*, 1908, xx, 793.
18. Stewart, H. A., *Jour. Exper. Med.*, 1911, xiii, 187.
19. Morgagni, J. B., De sedibus et causis morborum, etc., Venice, 1761, i, Letter IX, Art. 7, and Letter LXIV, Art. 5.
20. Spens, T., *Med. Comment*, Edinburgh, 1792–3, decade 2, vii, 458.
21. Adams, R., *Dublin Hosp. Rep.*, 1827, iv, 396.
22. Stokes, W., *Dublin Jour. Med. Sc.*, 1846, ii, 73.
23. His, W., Jr., III Internat. Cong. of Physiol., 1895; *Centralbl. f. Physiol.*, 1895, ix, 469; *Deutsch. Arch. f. klin. Med.*, 1899, lxiv, 316.
24. Gibson, J. R., *London Med. Gaz.*, 1838–9, xxiii, 123, 155.
25. Peacock, T. B., *Provincial Med. Jour.*, 1842, iv, 68; *Med. Times and Gaz.*, 1864, i, 7, 31, 56.
26. Cain, D. J. C., *Charleston Med. Jour. and Rev.*, 1849, iv, 271.
27. Rogers, Goddard, *Assoc. Med. Jour.*, 1856, iv, 1049; *Canstatts Jahresb.*, 1857, i, 199.
28. Cornil, *Gaz. méd. de Paris*, 1875, Ser. 4, iv, 292.
29. Blondeau, Thèse de Paris, Versailles, 1879.
30. Robinson, G. C., *Bull. Ayer Clin. Lab.*, 1907, No. 4, 1.
31. Gibbings, A. T., *Lancet*, 1885, i, 288.
32. Frey, A., *Berl. klin. Wchnschr.*, 1887, xxiv, 72.
33. Sendler, *Centralbl. f. klin. Med.*, 1892, xiii, 642.
34. Dumas, A., Thèse de Lyon, 1908, 316.
35. Rendu, *Bull. et mém. Soc. méd. d. hôp. de Paris*, 1895, xii, Ser. 3, 380; *Semaine méd.*, 1895, xv, 212.

36. von Starck, W., *Monatschr. f. Kinderheilk.*, 1903, ii, 11.
37. Bassoe, P., *Tr. Chicago Path. Soc.*, 1901–03, v, 247; *N. Y. Med. Jour.*, 1903, lxxviii, 445.
38. Dufour, H., *Bull. et mém. Soc. méd. d. hôp. de Paris*, 1904, Ser. 3, xxi, 639..
39. Handford, H., *Brit. Med. Jour.*, 1904, ii, 1745.
40. Debove, *Gaz. d. hôp.*, 1905, lxxviii, 135.
41. Medea, E., *Progrès méd.*, 1905, Ser. 3, xxi, 65.
42. Stengel, A., *Am. Jour. Med. Sc.*, 1905, cxxx, 1083.
43. Chapman, C. W., *Lancet*, 1906, ii, 219.
44. Keith, A., and Miller, C., *Lancet*, 1906, ii, 1429.
45. Keith, A., and Flack, M. W., *Lancet*, 1906, ii, 358.
46. Hay, J., and Moore, S. A., *Lancet*, 1906, ii, 1271.
47. Gibson, G. A., *Brit. Med. Jour.*, 1906, ii, 1113.
48. Barr, J., *Brit. Med. Jour.*, 1906, ii, 1122.
49. Aschoff, L., *Brit. Med. Jour.*, 1906, ii, 1103.
50. Brouardel, G., and Villaret, M., *Arch. de méd. expér. et d'anat. path.*, 1906, xviii, 230.
51. Schmoll, E., *Deutsch. Arch. f. klin. Med.*, 1906, lxxxvii, 554.
52. Fahr, *Virchows Arch. f. path. Anat.*, 1907, clxxxviii, 562.
53. Oddo and Sauvan, *Marseilles méd.*, 1907, xliv, 443.
54. Vaquez and Esmein, *Bull. et. mém. Soc. méd. d. hôp. de Paris*, 1907, Ser. 3, xxiv, 78.
55. Störck, *Semaine méd.*, 1907, xxvii, 324.
56. Butler, G. R., *Am. Jour. Med. Sc.*, 1907, cxxxiii, 715.
57. Ashton, T. G., Norris, G. W., and Lavenson, R. S., *Am. Jour. Med. Sc.*, 1907, cxxxiii, 28.
58. Jellinek, E. O., and Cooper, C. M., *Brit. Med. Jour.*, 1908, i, 796.
59. Jagic, N., *Ztschr. f. klin. Med.*, 1908, lxvi, 183.
60. Turrell, W. J., and Gibson, A. G., *Brit. Med. Jour.*, 1908, ii, 1486.
61. Gerhardt, D., *Deutsch. Arch. f. klin. Med.*, 1908, xciii, 485.
62. Gibson, A. G., *Quart. Jour. Med.*, 1907–08, i, 182.
63. Karcher, J., and Schaffner, G., *Berl. klin. Wchnschr.*, 1908, xlv, 1266.
64. Vickery, H. F., *Boston Med. and Surg. Jour.*, 1908, clix, 435.
65. Beck, H. G., and Stokes, W. R., *Arch. Int. Med.*, 1908, ii, 277.
66. James, W. B., *Am. Jour. Med. Sc.*, 1908, cxxxvi, 469.
67. Beeson, C. F., *Jour. Am. Med. Assn.*, 1908, l, 188.
68. Heineke, A., Müller, A., and von Hösslin, H., *Deutsch. Arch. f. klin. Med.*, 1908, xciii, 459.
69. Bramwell, B., *Brit. Med. Jour.*, 1909, i, 995.
70. Handwerck, C., *München. med. Wchnschr.*, 1909, lvi, 916.
71. Nagayo, M., *Zeitschr. f. klin. Med.*, 1909, lxvii, 495.
72. Gibson, G. A., and Ritchie, W. T., *Lancet*, 1909, i, 533.
73. Souques and Chéné, *Semaine méd.*, 1909, xxix, 59.
74. Magnus-Alsleben, E., *Zeitschr. f. klin. Med.*, 1910, lxix, 82.
75. Bishop, L. F., *Am. Jour. Med. Sc.*, 1910, cxxxix, 62.
76. Krumbhaar, E. B., *Arch. Int. Med.*, 1910, v, 583.
77. Barié, E., and Cléret, M., *Arch. d. mal. du coeur*, 1910, iii, 209.
78. Fleming, G. B., and Kennedy, A. M., *Heart*, 1910, ii, 77.

79. Pribram, A., and Kahn, R. H., *Prag. med. Wchnschr.*, 1910, xxxv, 233.
80. Koch, W., *Berl. klin. Wchnschr.*, 1910, xlvii, 1108.
81. Beck, H. G., and Stokes, W. R., *Jour. Am. Med. Assn.*, 1910, lv, 1065.
82. Armstrong, H., and Mönckeberg, J. G., *Deutsch. Arch. f. klin. Med.*, 1911, cii, 144.
83. Cohn, A. E., Holmes, G. M., and Lewis, T., *Heart*, 1911, ii, 241.
84. Monrad-Krohn, G.-H., *Arch. d. mal. du coeur*, 1911, iv, 350; *Norsk. Mag. f. Lægevidensk.*, 1911, ix, Suppl., 1.
85. Mollard, J., Dumas, A., and Rebattu, F., *Arch. d. mal. du coeur*, 1911, iv, 298.
86. Butterfield, H. G., *Heart*, 1912, iii, 203.
87. Price, F. W., and Mackenzie, I., *Heart*, 1912, iii, 233.

EXPLANATION OF PLATES.

Plate I.

In all the tracings, the upper curve is a record of the auricular movements; the lower curve that of the ventricular movements; upstrokes represent contractions. The time record gives tenths of seconds. The lowest line is that of the signal magnet.

Fig. 1. The first part of the tracing shows the action of the heart with vagi intact. The arrow marks the moment at which the right vagus was cut.

Figs. 2 and 3. Stoppage of the auricles on stimulation of the peripheral end of the right vagus. The ventricles are apparently not influenced.

Fig. 4. Effect of stimulation of the peripheral end of the right vagus during the cardiac acceleration that follows the administration of strophanthin. The auricles are completely inhibited; the only effect on the ventricles is a momentary disappearance of the extrasystoles.

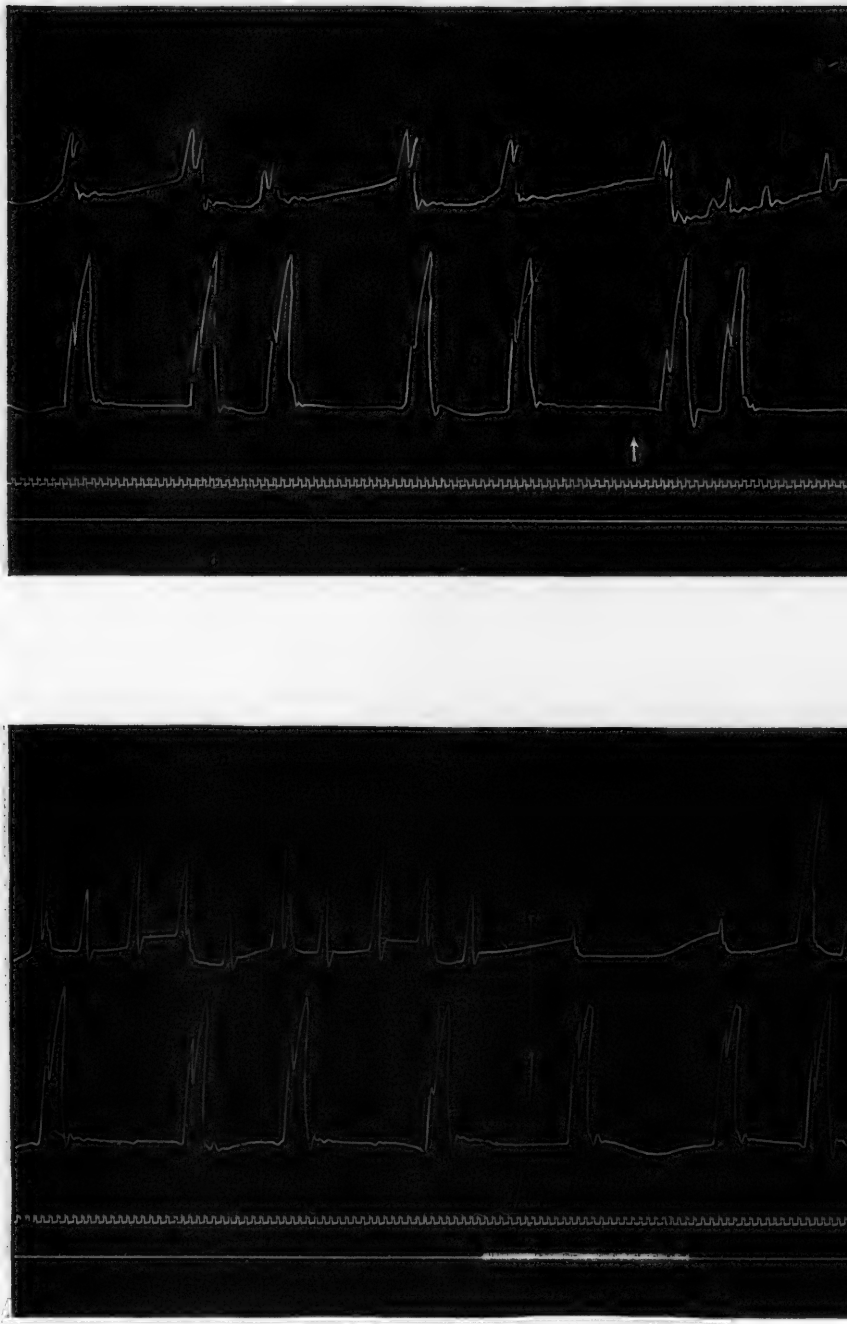


FIG. 3.

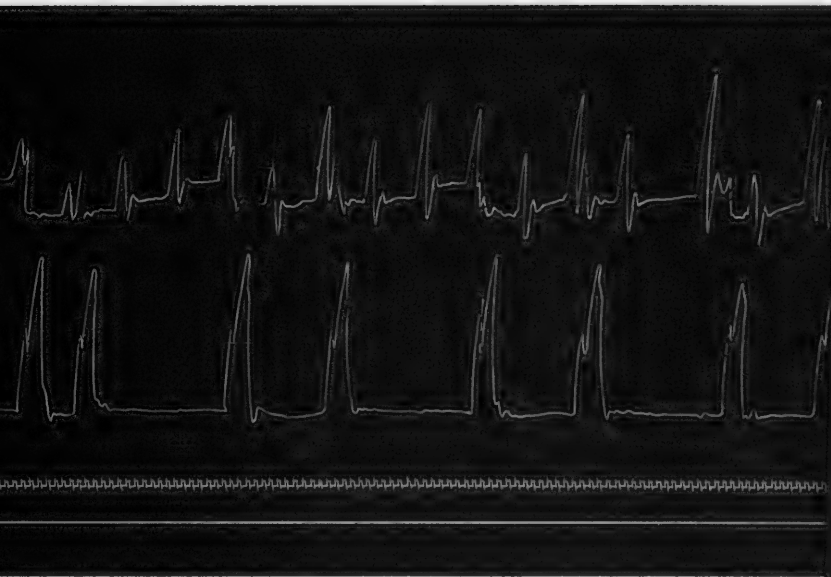
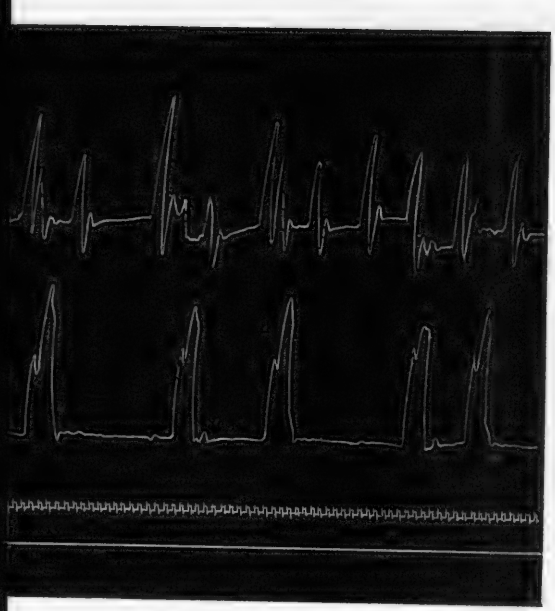


FIG. 1.



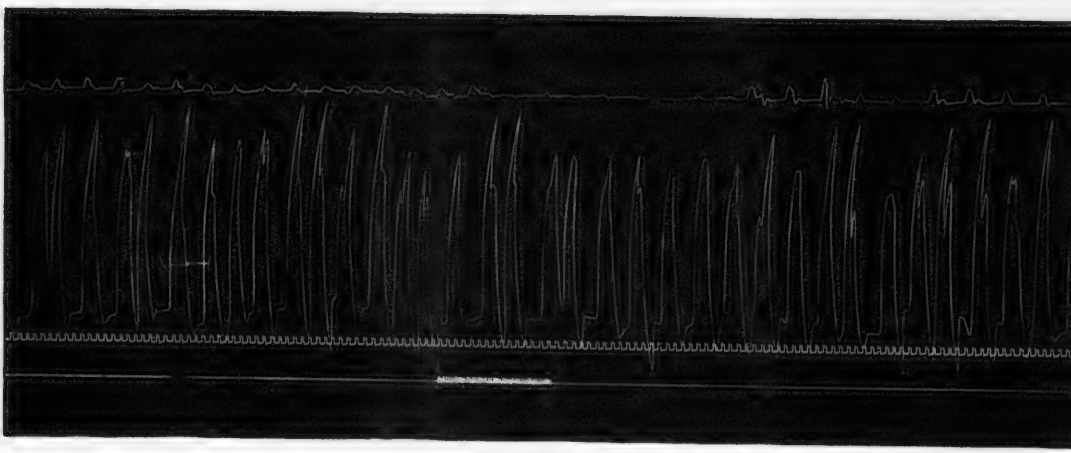


FIG. 4.

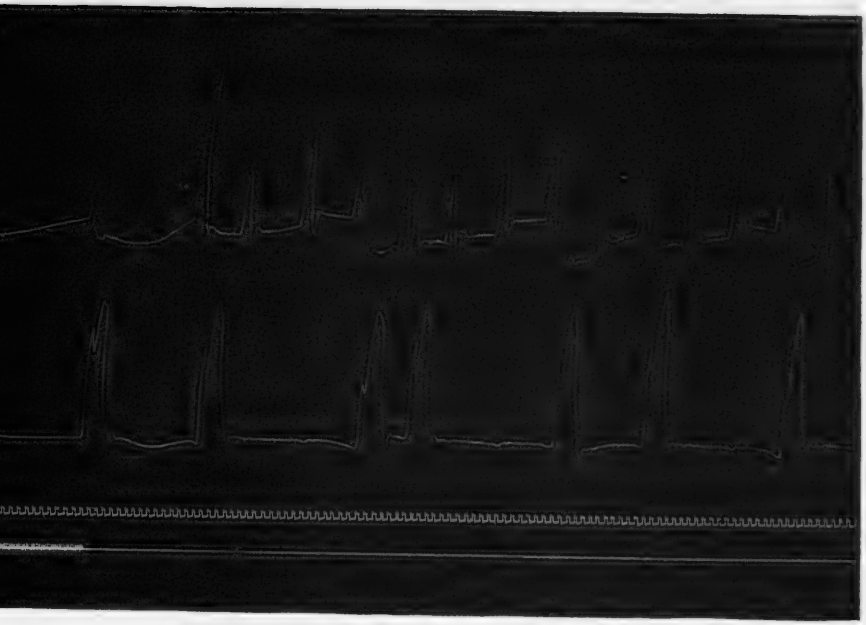


FIG. 2.

PLATE 2.

FIG. 5. First effect of strophanthin. The transformation of a relatively complete, into an absolutely complete block by abolishing all a-v conduction. The ventricles beat regularly.

FIG. 6. Further action of strophanthin. Irregular inhibition of the auricles by stimulation of the cardio-inhibitory center. The ventricles continue to beat regularly at their own rhythm.

FIG. 7. Further action of strophanthin. The auricular rate is increased The lower record shows the beginning of the increase in ventricular rate.

FIG. 8. Further action of strophanthin. The increase in auricular and ventricular rates is well established.

FIG. 9. Further action of strophanthin. Group beating of the auricles. The ventricular rate is notably increased. The vigor of the contractions is greater than at any other time during the experiment.

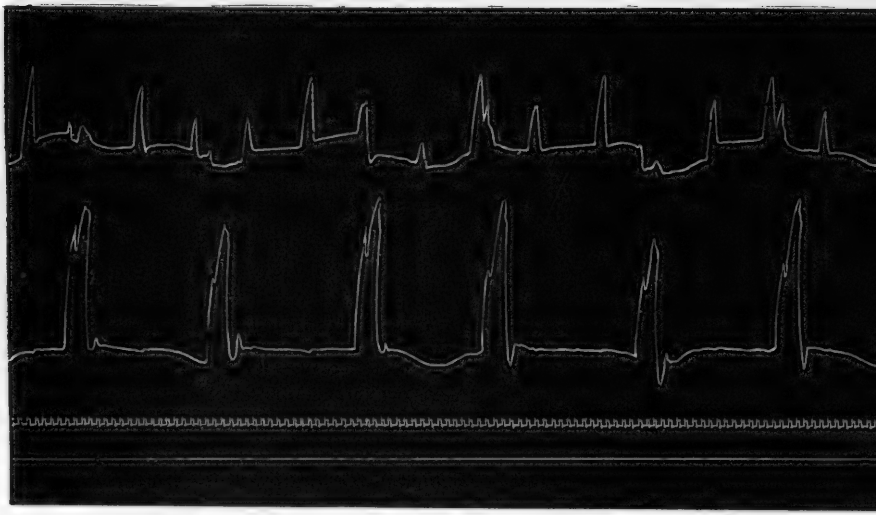


FIG. 5.

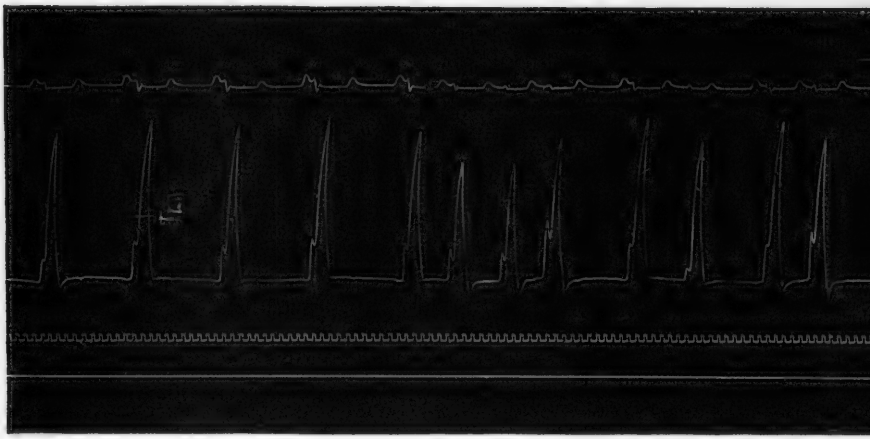
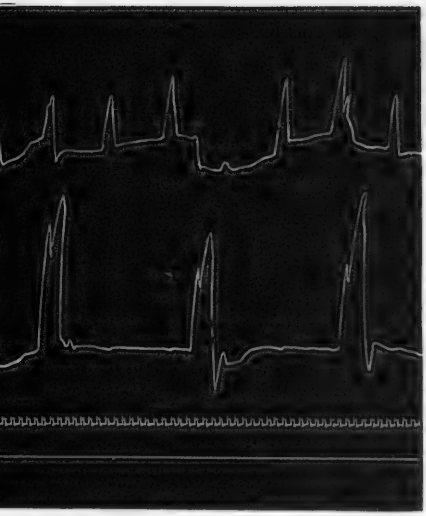
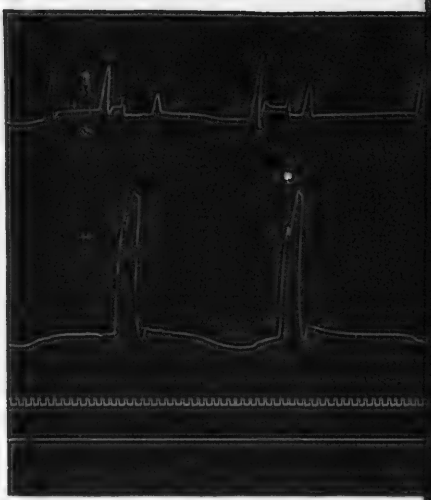


FIG. 7.





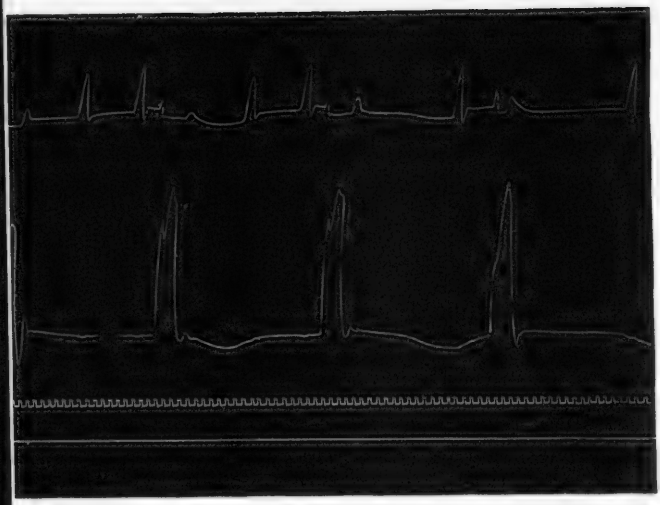


FIG. 6.

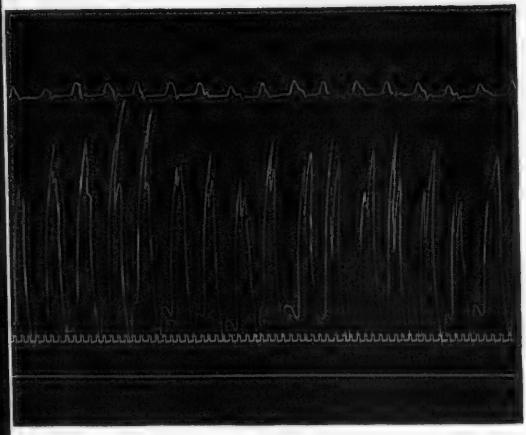
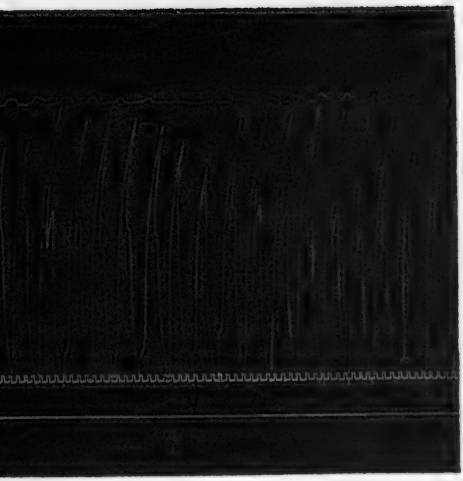


FIG. 8.

F

PLATE 2.



. 9.

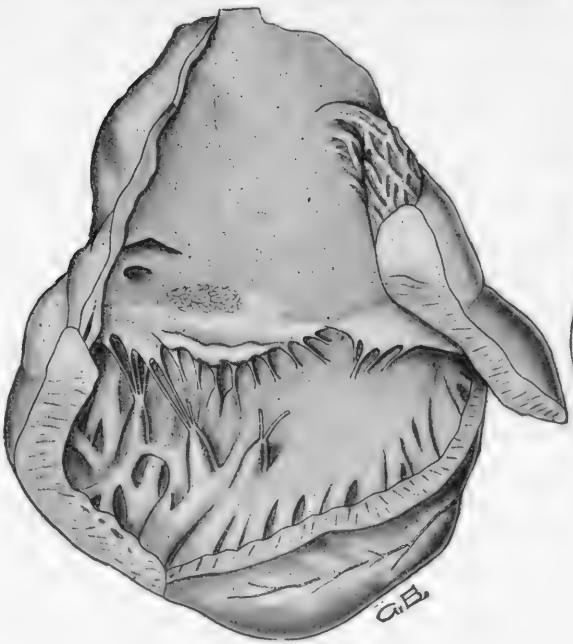


FIG. 10.

FIG. 11.

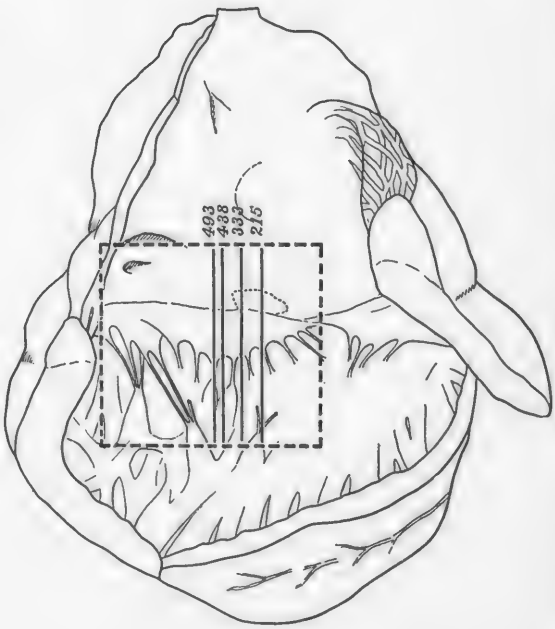


FIG. 12.

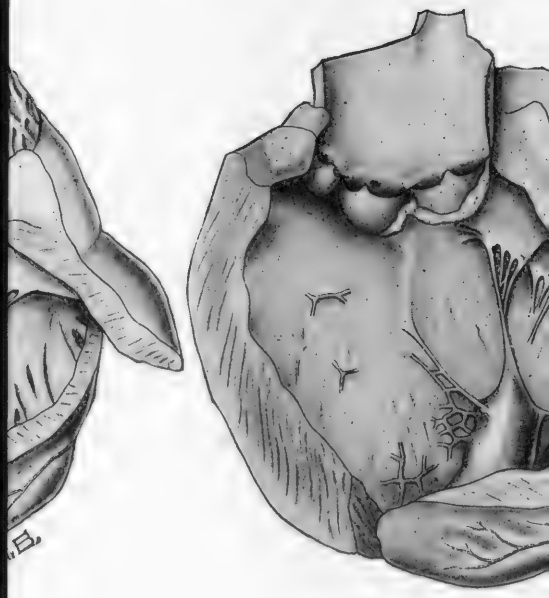


FIG. 11.

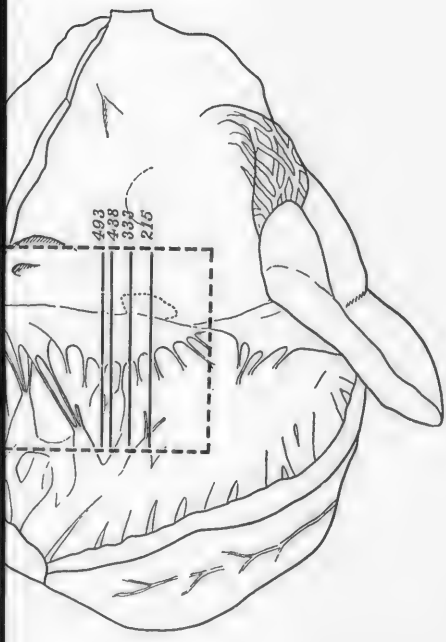


FIG. 12.

PLATE 3.

FIG. 13.

FIG. 14.

FIG. 15.

FIG. 16.

PLATE 3.

FIG. 10. Right side of the septum (natural size). Note the swelling at the free margin of the septal tricuspid leaflet and the grayish patch with distended capillaries above the attached margin of the same leaflet. The light area in front of the patch indicates the position of the pars membranacea septi.

FIG. 11. Left side of the septum (natural size). Note the swelling at the attached portion of the right anterior and of the posterior aortic leaflets. The nodule at the angle between these leaflets overlies the point of division of the a-v bundle. The left limb of the bundle shows as a light band apparently continued into the false tendon running into the papillary muscle.

FIG. 12. Diagram of the right side of the septum showing the size and position of the block of tissue examined in serial sections. The numbered vertical lines indicate the position of the sections here illustrated. It will be seen that two of these include the pars membranacea septi (in dotted outline); the other two are in the region of the main body of the bundle.

PLATES 4–7.

FIGS. 13, 14, 15, 16. Camera lucida drawings of sections 215, 333, 438, and 493, respectively. Their position in the septum is indicated in figure 12.

a = auricular muscle; v = ventricular muscle; f = fibrous septum; b = a-v bundle; rl = right limb; ll = left limb; n = new fibrous tissue (myxomatous?); d = right side of fibrous septum; s = left side of fibrous septum; c = aortic cusp; t = tricuspid leaflet; w = aortic wall.

STUDIES ON PNEUMOCOCCUS INFECTION IN ANIMALS.

First Paper.*

By AUGUSTUS B. WADSWORTH, M.D.

(*From the Department of Bacteriology of the College of Physicians and Surgeons, Columbia University, New York.*)

INTRODUCTION.

Lobar pneumonia is simply one phase of pneumococcus infection. Previous research has clearly established this even if it has failed to secure practical results in either prevention or cure of the disease. In order to understand adequately the conditions underlying the development, the prevention, and the cure of the disease, tradition, which has so long considered the lobar pneumonia of man to be a disease entity by itself, must be set aside and the broader conception of it as one form only of pneumococcus infection must be fully realized.

Experimental Pneumonia.—The results of the early attempts to induce lobar lesions in animals proved so unsatisfactory that in 1901, when the writer (1) first turned to the study of experimental pneumonia, the significance of the results of previous observers was not apparent. By experiments on the rabbit with the pneumococcus in which the conditions of tissue susceptibility and bacterial virulence were accurately controlled, varied, and balanced one against the other, it became evident, that it was not wholly the bacterial incitant, but the susceptibility of the host as well, that determined whether the disease processes would be bronchopneumonic, lobar, or bacteriemic. In the light of these studies the failure of previous observers to obtain lobar lesions in susceptible animals, such as the mouse, guinea pig, and rabbit, became significant, and the importance of the relatively few successful experiments of Gamaléia (2), in 1888, and of Tschistovitsch (3), in 1890, on more resistant animals such as the dog, were emphasized. These successful experiments Welch (4), in 1892, was unable to confirm, but they have been fully verified by the convincing results recently secured by Lamar and Meltzer (5).

The results of the researches of the writer and those of previous observers were fully recorded in 1904, and it is not necessary to review them here in detail. Let it suffice to say that this study clarified the broader conception of

* Received for publication, March 28, 1912.

the etiological conditions underlying pneumococcus infection in general and of lobar pneumonia in particular.

In man, pneumococcus infection of the lung takes the lobar form; in very susceptible animals, such as the rabbit, the experimental infection gives rise to comparatively little lung reaction, and is chiefly bacteriemic, but when partially immunized these animals may develop extensive lesions of the lung. In man it is the exceptional conditions of an exalted virulence of the pneumococcus in the presence of a relative insusceptibility that determines the lobar form of the disease, characterized as it is by a specific or limited bacterial etiology and by distinct anatomic and symptomatic manifestations.

Recovery.—Previous study of the phenomena of recovery in the lobar pneumonia of man and in pneumococcus infection of animals has failed to reveal any fundamental differences. Even crisis, which in no other disease and in no other form of pneumococcus infection is so marked, so complete, or so frequent, is, after all, simply sudden recovery, and all attempts to discover in the crisis something apart from recovery in the ordinary sense have one and all failed. Nevertheless all of this study has contributed much valuable information to our knowledge of pneumococcus infection.

Pneumococcus Immunity.—The most significant results have been derived from the study of the protective mechanism in pneumococcus infection. Following the discovery of the relationship of the pneumococcus to pneumonia by Fraenkel (6) in Germany, Talamon (7) in France, and Salvioli (8) in Italy, these observers and many others, notably Foa and Bonome (9), Foa and Carbone (10), Behring and Nissen (11), Bonome (12), Foa and Bordonni-Uffreduzzi (13), Emmerich and Fowitsky (14), Kruse and Pansini (15), Tschistovitsch (3), and Arkharow (16), recognized and studied pneumococcus immunity. These and other more recent studies on the phenomena of immunity in general and of pneumococcus immunity in particular have revealed the several processes active during recovery although they have failed to establish their relationship to each other, or the practical significance of the protective mechanism as a whole.

A. Humoral Activities.—Theories of toxin and antitoxin neutralization were first suggested by Behring's studies in diphtheria and Kitasato's in tetanus, both of which studies were then new.

Behring and Nissen noted a slight bactericidal action on the pneumococcus in the blood sera of the more resistant animals after immunization, but in the blood sera of very susceptible animals this was even less marked. These observers considered pneumococcus immunity to be chiefly antitoxic and quite similar to that of tetanus and diphtheria. Similarly, by precipitation of forty-eight hour cultures of the pneumococcus, G. and F. Klemperer (17) obtained substances which were toxic in large doses. The toxic action of this "pneumotoxin" was neutralized by sera from immunized animals. These sera possessed also a curative action on mouse infections, but gave less definite results when used in the lobar pneumonias of man. Mosny (18) also concluded that the immunity in pneumococcus infections was due to antitoxins, and the recent studies of Tizzoni and Panichi (19) revert to these early interpretations of the action of pneumococcus immunity. But no true toxin has been demonstrated, and the presence of antitoxin has only been inferred. Hence, theories of toxin and antitoxin neutralization have aroused little interest in modern research.

The failure to find a soluble toxin in pneumococcus cultures has fostered the belief that the active substances were endotoxins. The endotoxins of cholera, typhoid, and many other organisms were readily demonstrated, but those of the pneumococcus have proved more elusive. In a recent and very complete summary of previous research, Kruse (20) concludes that active poisons from the pneumococcus have not been secured.

More recently, however, Rosenow (21) has described the toxic action of substances obtained at certain stages of the digestion of sera with autolyzed pneumococcus material. His results are extremely suggestive in the light of similar work by Dellezenne and Ledebt (22) on cobra venin. At certain stages of the digestion of sera with cobra venin substances were formed that proved 10,000 times as toxic as the venin alone. These observations not only open to investigation a large and extremely important field, but they broaden our conceptions of hypersusceptibility and of the action of toxin and endotoxin in the animal organism, so that in the light of future study, these may be brought into a closer and truer relation.

The significance in pneumonia of the phenomena first noted by Theobald Smith (23) and later more fully described by Rosenau and Anderson (24) as anaphylaxis has not as yet been fully determined. The experiments of Friedemann (25) and Friedberger (26), by showing that complement and immune body are consumed in anaphylaxis, suggest that this phenomenon is after all simply a phase of the intracellular chemistry of infection bearing on the action of toxins and endotoxins.

The distinction of toxin from endotoxin has been convenient and is generally although tentatively accepted, but whether or not it is fundamental will be decided when the chemistry of bacterial poisons is better known. That the differences are not necessarily fundamental is now more fully realized than it was when the distinctions were first drawn. Hence Bail's (27) attempts further to distinguish aggressins have received scant notice, despite Römer's (28) exposition of aggressin action in its relation to pneumococcus infection of the eye.

The failure to obtain significant results in the neutralization of pneumococcus poisons by the body fluids has encouraged the belief that the mechanism of recovery is chiefly bacteriolytic.

Bactericidal action was noted in certain sera by Behring and Nissen, and was considered an active factor in the destruction of the pneumococci during recovery. Foa, Carbone, Emmerich and Fowitsky, and Arkharow, all held similar views but failed to offer any very convincing experimental demonstration in support of their conclusions. Kruse and Pansini, in 1892, however, found that the pneumococcus when incubated at 37° C. developed as rapidly in immune as in normal sera. Nevertheless, Welch (29) in 1890 noted lysis of the pneumococcus within its capsule. This was described in more detail ten years later by Radziewsky (30) who used the Pfeiffer technique, and by Neufeld (31) who tested immune serum in the hanging drop.

Although the discoveries of Bordet (32) and Landsteiner (33) and the ingenious theories of Ehrlich have contributed much significant knowledge regarding the action of amboceptor and complement in the phenomena of bacteriolysis in general and of the cholera and typhoid organisms in particular,

comparatively little is known about the bacteriolysis of the pneumococcus save that it may be seen under the microscope and takes place in the tissues.

To what extent the results of tests with sera *in vitro* may be accepted as significant in interpreting what occurs in the tissues has never been definitely determined. Metchnikoff (34) claims that cytase does not exist free in the circulating plasma, but that it is liberated after injury or death of the leucocyte. There is a long list of observations, which it is scarcely necessary to review. Certain of these show that lysis of foreign red cells injected into the veins of immunized animals give rise to hematuria and then suggest the presence of cytase in the plasma. Other experiments, however, indicate that cytase is not present in the aqueous humor until after injury of the eye, and the experiments of Pettersson (35) and others show that in plasma obtained from centrifugalized blood, cytase is absent or present in small quantities only. None of these experiments, however, accurately represent conditions actually present in the circulating blood during infection.

Cytase may not be present in the circulating blood plasma under normal conditions, but in infectious disease there is a varying amount of injury. It may well be that in immunity, the chemical affinities between amboceptor—known to be present in the plasma—and its antigen, the bacterial cell, suffice to free the cytase which under normal conditions is held in the cells. Whether or not this liberation of cytase would constitute an injury in Metchnikoff's sense is a question of no great practical importance at present.

In the experiments of Hoessli (36) and of Dold (37), whole blood and blood plasma were definitely more bactericidal for the pneumococcus *in vitro* than the corresponding sera. Whether the reactions concerned were those of specific amboceptor and complement was not determined.

Although complete experimental evidence of the action of amboceptor and complement is lacking, lysis of the pneumococcus as described by Welch, by Radziewsky, and by Neufeld, resembles very closely that of other bacteria and there is no reason to believe that a fundamental difference exists.

B. Cellular Activities.—Kruse and Pansini found that although the pneumococcus in the test-tube grew readily in immune sera, in the tissues during recovery its growth was inhibited and the pneumococcus cells were taken up by the phagocytes. In their experiments this phagocytosis was increased by the introduction of immune sera. Both Mennes (38) and Issaeff (39) (in Metchnikoff's laboratory) found that immune sera favored phagocytosis. Issaeff thought this was due to the action of substances stimulating the leucocytes to greater activity, a view now supplanted by the more accurate conceptions of opsonification which originated in the work of Denys and Leclef (40). These conceptions have been more fully elucidated by the work of Wright and Douglas (41), of Neufeld (42), and of Hektoen (43). These observers have shown that immune serum acts on the bacterial cell rendering it more subject to phagocytosis, that the reaction is to a certain extent specific, and that it is also complex, since complement is to some extent a factor.

Virulent strains of the pneumococcus, however, were found to be quite as insusceptible to the opsonic action of immune serum, as they were to the bactericidal action. From cultures of other organisms Van de Velde (44), Neisser and Wechsberg (45), Bail (46), von Lingelsheim (47), and others

obtained the so-called "leucocidins" which were toxic for the leucocytes. Similarly from virulent pneumococci both Rosenow (48) and Tschistovitsch (49) obtained extractions which they described independently as "virulins" and "antiphagins" and to the presence of which they ascribe the failure of the leucocytes to take up virulent strains of the pneumococcus. Non-virulent pneumococci and the virulent pneumococcus cells after extraction under certain conditions were found to be subject to the opsonic action of serum. But as might well be expected the study of phagocytosis under these conditions has proved of doubtful significance in interpreting the conditions underlying the phagocytosis of the pneumococcus in infectious processes.

Wright's ingenious methods opened up the promising study of the opsonic activity of the serum of pneumonia patients, and with this subject research has recently had much to do. Neufeld (50), Rosenow (51), Potter and Krumwiede (52), and others have found that the opsonic activity of the blood serum and the phagocytic activity of the leucocytes were increased in pneumonia, but Seligmann and Klopstock (53), Boettcher (54), and Strouse (55) were unable to confirm the claim that this increase is a material one.

Insuperable technical difficulties account for much variation in the determination of opsonic indices. These Wright and Hektoen have not fully recognized. But North (56), Park (57), and others have demonstrated many of the errors in the study of the opsonic index at the bedside.

The writer's suspicions were first aroused when normal serum seemingly proved more actively opsonic for the staphylococcus than that of a highly immunized animal. By experiment, this was found to be due to the agglutinative action of the immune serum and the writer called attention to this error at the meeting of the Roosevelt Alumni Association of New York, in January, 1907.[1] Similarly at a meeting of the Association of American Physicians in June, 1907, Baldwin noted the fact that the agglutinative action of sera was a

[1] A very dense suspension of staphylococci was added to mixtures of normal and immune sera with the leucocytes in equal quantities and incubated at 37° C. The phagocytic index of the normal serum was 5.18 in ten minutes, and 10.86 in twenty minutes. That of the immune serum was 2.22 in ten minutes, and 23.66 in twenty minutes.

These results were due to the agglutination present in the immune sera. As shown by examination of the smears the clumping at first interfered with phagocytosis, then later greatly facilitated it, because the clumping often took place about the leucocytes.

It is not easy to estimate accurately the number of organisms the phagocytes take up. In the mixtures of leucocytes, bacteria, and immune sera, bacteria will stick to the leucocytes. This may be due to chance contact or to agglutinative action, but it is of frequent occurrence and cannot be accurately distinguished from phagocytosis. Bacteria in vacuoles of the cytoplasm and bacteria undergoing degeneration are readily recognized as intracellular, but when the leucocytes are well spread out and dried, and when the cytoplasm is faintly stained as a thin film, it is impossible to determine whether the bacteria are inside, above, or below the cell. These are real difficulties and they are here emphasized because they have received scant notice.

factor in estimating opsonic indices with tubercle bacilli. But North, at the same meeting, presented statistics showing the magnitude of the error. He sent to ten laboratories active in opsonic work sera known only to himself, and through their coöperation found that the error of these experienced workers averaged 45.5 per cent. The minimum error was 24 per cent.

Since these early investigations, the technique has been improved. This has doubtless diminished the error, but it has also rendered the method laborious without correcting the more serious difficulties. Hence the significance of the opsonic index is doubtful at best and the study of it has failed to add to our knowledge of phagocytosis in pneumonia. Study of the lung exudate, however, has given much more significant results.

Tschistovitsch (3), in 1904, found that in the experimental pneumonia of the dog phagocytosis was marked. He cites the early experiments of Patella (58) who, by paracentesis of the pneumonic lung of man, found that the pneumococci disappeared very rapidly and were rarely present after the crisis. Thus, Tschistovitsch thought the crisis was due to phagocytosis, and many of the more recent observers favor this view.

Other phases of cellular immunity more or less closely related to phagocytosis have been considered in their relation to the recovery from pneumococcus infection. Substances of a bactericidal nature have been often found in the leucocytes; for example, in Buchner's (59) early work and in the recent investigations of Pettersson (60), Wassermann (61) demonstrated the fact that of all the tissues of the immunized animal the bone marrow was the most actively protective against pneumococcus infection. Hiss (62), from his study of the protective and curative action of aqueous extracts of rabbit leucocytes, suggests that pneumococcus immunity is due to the presence of protective substances in the body cells which are liberated only under certain conditions and are not usually present in the body fluids.

After reviewing previous work Flexner (63) turns for new light to another phase of the intracellular chemistry of infection, introducing Lamar's study of the action of the unsaturated fatty acids and alkaline soaps present in exudates. Lamar (64) found that the presence of these substances in the test-tube and in the tissues during infection increased the action of immune sera on the pneumococcus, but the practical significance of this is not as yet apparent.

Up to the present, research has wavered between humoral and cellular conceptions of immunity, turning first to one then to another of the various phenomena of pneumococcus infection in the attempt to determine the conditions underlying recovery in order that practical results might be achieved in the cure of pneumonia in man. Focused on a part, this study has overlooked the protective mechanism as a whole in its broader and simpler phases, but out of this chaos some order must be established.

I have carried out many closely related experiments, but give in this paper only the ones that deal with the action of pneumococcus

cultures on animal tissues, and the action of immune sera on the pneumococcus.

ACTION OF THE PNEUMOCOCCUS ON ANIMAL TISSUES.

The several types of tissue reaction which living cultures of the pneumococcus incite in different animals have been too fully described in the experiments of others and in those of the writer (1) to warrant further consideration.

Tissue reaction, whether in the form of local lesions or of cellular injury throughout the animal organism, is due to the poisons of the infectious agents. These poisons may be set free in soluble, diffusible form, or they may be so fixed in the bacterial cell that they become soluble only after lysis has taken place.

Dead Cultures.—In order to determine the nature of the pneumococcus poisons, attempts were made to induce the types of lesions obtained with living pneumococci by the introduction of dead culture material. Lesions of this kind have been produced by the introduction of the dead cultures of certain organisms. Roux and Yersin (65), Behring (66), and Kitasato (67) induced characteristic disease processes by the inoculation of culture filtrates free from tetanus and diphtheria organisms. With dead tubercle bacilli Prudden (68) and others obtained typical tubercular lesions in the lung.

In my experiments culture filtrates and masses of dead pneumococcus cells were injected into the veins, under the skin, intraperitoneally, and through the trachea of rabbits.

Whereas intravenous and subcutaneous inoculations of dead typhoid, dysentery, cholera, and other organisms have proved highly toxic and often fatal, pneumococcus material, in my experiments as in those of others, gave rise to little reaction in the animal organisms.

Culture filtrates of the most virulent organisms in doses of ten cubic centimeters caused little or no disturbance. The animals invariably made an immediate recovery which differed apparently in no respect from that of control animals that received similar quantities of sterile uninoculated broth. And yet, following the introduction of these culture filtrates of the pneumococcus, the ani-

mals developed an immunity to virulent pneumococci not present in the controls.

Concentrated Culture Material—Dense suspensions of pneumococcus cells proved but little more toxic than the culture filtrates. Evanescent temperature reactions were seldom noted. Continued frequent inoculation of this material caused emaciation and ultimately death, but no significant lesions were found at autopsy. Usually, however, the animals recovered rapidly after these inoculations and were then immune to virulent living cultures. Although the concentrated cultures gave rise to more disturbance in the animal organism, the culture filtrates incited more quickly and more certainly the immunity to living cultures.

In order to measure the degree of immunity incited after intravenous, subcutaneous, and intraperitoneal inoculation, the following experiment was made with dead culture material:

Six rabbits received an immunizing dose of 5 c.c. of pneumococcus culture material;[2] two were inoculated intravenously, two subcutaneously, and two intraperitoneally. Eight days later one animal of each set was inoculated intravenously with 1 c.c. of virulent culture, the other with 0.5 c.c. At the same time two normal rabbits were similarly inoculated.

The controls died in less than twenty hours, but both of the animals immunized by intravenous inoculation survived, the virulent culture causing little or no disturbance in these animals. The animals immunized by subcutaneous inoculation died, one in three days, the other in eleven. Of the two immunized by intraperitoneal inoculation, one died and the other recovered. Intravenous inoculation was, therefore, much the most effective in producing immunity, but neither intravenous nor subcutaneous inoculation of dead pneumococcus culture material gave rise to significant lesions in the animal tissues.

Intratracheal Injection.—Owing to its structure the lung is apparently as well adapted for the accumulation of exudate as it is to its absorption. In order to determine the effect of dead pneumococcus culture material on the lung tissues intratracheal injections were made.

Five to ten cubic centimeters, depending on the size of the rabbit, is the maximum quantity that can be injected into the lung through

[2] This material was obtained by precipitating a twenty-four hour pneumococcus broth culture with ammonium sulphate. The precipitate after complete dialysis became partially soluble, leaving only a little suspended material in the fluid. This fluid was filtered through a Berkefeld candle and the filtrate used for the immunization.

the trachea without regurgitation or great danger of a contaminating infection from the upper air passages.

In a series of rabbits, 5 c.c. of virulent culture, heated to 52° C. for thirty minutes to kill the pneumococci, failed to incite lung lesions. In fact the absorption of the foreign material and the return of the tissue to normal took place more quickly in the lung than in the subcutaneous tissues.

In another experiment (March 4, 1901) I injected intratracheally culture sediment and, for purposes of comparison, the decanted clear fluid of these cultures. The 6 rabbits receiving 10 c.c. of fluid free from pneumococcus cells, were killed on the third and fourth days. The lungs were normal. The 6 rabbits receiving 2, 2, 2, 3, 3, and 8 c.c. of the dead pneumococcus cells, were killed and autopsied two and four days after the intratracheal injection. The lungs and viscera were sterile. Irregular areas of bronchopneumonic consolidation were found about the large bronchus of the left lower lobe, to which the fluid had been directed. The extent of lung reaction depended upon the quantity injected. Microscopical examination of the lung showed atelectasis, induration with cellular infiltration of the walls of the air spaces, and some little exudation and exfoliation of cells into the air spaces; in short, a foreign body pneumonia.

In testing the power of the lungs to dispose of various solutions and suspensions, 10 rabbits (October 23, 1901) received intratracheal injections of 5 c.c. each of the following substances: sterile 3 per cent. peptone meat infusion broth, decanted culture fluid free from pneumococcus cells, culture sediment washed and suspended in salt solution, scrapings of a Berkefeld filter suspended in salt solution and sterilized; and a salt solution suspension of the scrapings of a filter that had been heated to 52° C. for thirty minutes after a liter of pneumococcus culture had passed through the filter. In forty hours the animals were killed and autopsied. The lungs and viscera were sterile and no significant lesions were found.

Neufeld (31) had found that rabbit's bile in a dilution of 1 to 20 parts of culture dissolved the pneumococcus cell. Accordingly concentrated mass cultures of pneumococcus cells were treated with bile in this dilution and injected through the trachea of 2 rabbits in doses of 7 and 8 c.c. Marked dyspnea developed rapidly in both animals and one died in twenty-four hours. The other recovered, but was killed and autopsied on the third day.

Marked lesions were found in the lungs of the animal that died. But they were also found in controls injected with dilutions of bile in sterile broth. The lungs and other organs of these animals were sterile.

Similarly extracts of the pneumococcus cell without bile were tested. A concentrated culture sediment was suspended in 17 per cent. salt solution. allowed to stand over night in the ice box, and was then diluted with 20 volumes of water and filtered. The filtrates contain specific precipitable substances as described in a former paper (69), but when injected through the trachea these extracts caused no disturbance whatever.

Summary of the Studies on the Action of Dead Pneumococcus Material on Animal Tissues.—From the results of these experi-

ments it is evident that dead cultures of the pneumococcus do not contain the active poisons elaborated by the organism in infection. In no instance by subcutaneous, intravenous, intraperitoneal, or intratracheal inoculation were characteristic lesions obtained. Nor was this dead culture material toxic to any demonstrable extent. And yet, the animals after inoculation acquired an immunity to pneumococcus infection, the immunity being more marked after intravenous inoculation.

From this it is apparent that pneumococcus cultures contain altered or degenerated poison, possibly comparable to the toxons or toxoids of diphtheria cultures, or substances, possibly enzymes, such as are present in snake venin, which when liberated in infection give rise to more powerful poisons, but when formed in culture are active only as antigen. These substances were present in the pneumococcus cells free from culture products, but the culture filtrates contained proportionately greater quantities. Apart from this, the results of these experiments add little to our knowledge of the pneumococcus poisons, and further light was obtained only indirectly from a study of pneumococcus immunity.

ACTION OF IMMUNE SERA ON THE PNEUMOCOCCUS.

That a protective mechanism brings about the recovery from pneumococcus infection is manifest, and in the crisis of lobar pneumonia in man this is particularly evident. And yet, in the previous studies of pneumococcus immunity immune sera have lacked bactericidal action, and phagocytosis was exceptional.

In the hope of determining experimentally why this is so, the following studies on agglutination, bacteriolysis, and phagocytosis of the pneumococcus were made.

Studies on Agglutination.—In a former paper (69) the technical difficulties and improved methods of securing agglutination of the pneumococcus in high dilution were fully recorded. By substituting fine suspensions of centrifugalized pneumococcus cells in normal salt solution for the ordinary broth cultures it was possible to obtain positive reactions even after diluting sera obtained from immunized animals and from pneumonia patients.

Forty-six cases of pneumonia were examined. These included one case complicated by empyema and one case of pneumonia in a patient with phthisis. For purposes of control, three cases of nephritis, three of gas poisoning, five of endocarditis and rheumatism, and one each of cerebrospinal meningitis, tonsillitis, and alcoholism were studied.

In all the controls except three, agglutination took place in a dilution of 1:10 or 1:15. Serum from one case of gas poisoning failed to agglutinate, but in another case and in the case of cerebrospinal meningitis, agglutination was noted in dilutions of 1:40.

In the sera from pneumonia patients the agglutination varied from 1:15 up to 1:300. The maximum agglutination was recorded in thirty-eight cases, and the average as estimated from these was 1:77. In fatal cases agglutination was occasionally low, but often it was above the average. In sera obtained after the crisis the agglutination was not significantly increased; in fact one of these agglutinated only in a dilution of 1:20. In both favorable and unfavorable cases the agglutination was below the average. Hence prognostic and diagnostic deductions could not safely be drawn from these results alone.

Pneumococcus agglutination in pneumonia, like that of the typhoid bacillus in enteric fever, varies greatly and in a similar way. It is, however, much less marked, and is, therefore, more difficult to determine accurately. But since in pneumonia bedside data usually suffice for both diagnosis and prognosis, there is little need of it for the present. But should an efficient serum therapy be secured, a specific bacterial diagnosis may be required in exceptional cases.

The results of this study of the agglutination of the pneumococcus in the blood serum of pneumonia patients were thus of comparatively little practical significance.

Studies on Bacteriolysis.—Two of the sera from the cases of pneumonia were markedly lytic. This, however, developed only in the lowest dilutions and was not present in the sera from any of the other forty-four cases. Accordingly I turned to the serum of immunized animals for a more satisfactory demonstration of the phenomena and the conditions under which it takes place. Lysis of

the pneumococcus, as described by Welch, by Radziewsky, and by Neufeld, was readily seen in the hanging drop of serum from a highly immunized rabbit. The encapsulated pneumococci swell, refract the light less and less, until finally the bacterial cell disappears within its capsule. On staining the cells with capsule stains, the capsules were found empty. This lysis, however, was rarely complete, and when studied at 37° C. was invariably followed by rapid growth of the surviving pneumococci.

The activity of the different sera varied and the cultures of pneumococci differed in susceptibility. Highly virulent organisms were less susceptible. One strain, which by passage from rabbit to rabbit had been under parasitic conditions for nearly two years, was exceptionally resistant to the lytic action of sera.[3] But no very precise estimate of this could be made.

The observer of lysis of the pneumococcus is forced to rely on impressions drawn from microscopical appearances rather than on figures. The presence of agglutination in this as in other tests of bacteriolysis is confusing when estimates are made by plating and counting colonies. But apart from this the pneumococcus often fails to grow in agar plates, and one strain, cited above, owing to its parasitic adaptation failed to grow even on the surface of agar plates, when no serum was present. Hence attempts to measure the variations in so fleeting and uncertain a phenomenon as lysis of the pneumococcus *in vitro* have failed.

Lysis of the pneumococcus in immune sera is slight compared with that of the organisms of cholera or of typhoid fever. This is scarcely due to structural peculiarities because in dilute rabbit's bile and even in culture the pneumococcus is exceptionally susceptible to lysis. It is in fact extremely sensitive to conditions which are in the least degree unfavorable. But in the blood sera conditions are evidently most favorable for the pneumococcus and growth takes place, whereas for the typhoid and chol-

[3] In one test of the bactericidal action of different sera upon this organism, the streaks on the surface of agar plate gave growth only in the immune and normal sera, the salt solution controls being sterile. As subsequently tested, using serum agar plates, slight bactericidal action was found in certain of the immune sera, but the destruction of the pneumococci was quite as marked in the salt solution controls.

era organisms sera are not so favorable. For these two types of organisms conditions are evidently reversed, and adaptation of the cell metabolism and growth seem to be the determining factors of lysis of the pneumococcus. Thus, under the ordinary conditions of procedure, my study of the lytic action of immune sera has been barren of significance.

In interpreting conditions present in infection, the significance of tests with sera *in vitro* has never been satisfactorily determined. This doubtless varies in the different bacterial infections. But it is certain that during infection lysis of the pneumococcus takes place in the tissues.

Following prolonged bacteriemia the rabbit often recovers by a crisis similar to that of man. Although it is of course difficult to be sure that a sudden fall in temperature with marked betterment in the animal's condition is really crisis, this was successfully diagnosed in a few instances, and the animals were killed when the temperature fell to normal. At autopsy few pneumococci were found and often the cultures were sterile. In two animals empty capsules and other signs of lysis, but with no evidence of phagocytosis, were found in the pericardial fluid.

After intravenous inoculation when the cultures are not excessively virulent, there is for a short interval a marked destruction of the pneumococci. This destruction is extracellular and entirely bacteriolytic. At least no signs of phagocytosis are to be found.

Evidently in the tissues, conditions are present which do not exist in the test-tube, for in the tissues the activity of the pneumococcus is inhibited in varying degree and during recovery lysis takes place. An attempt was accordingly made to secure in the test-tube conditions comparable with those in infection. Inasmuch as we may conceive that the development of the pneumococcus is retarded in infection by the febrile temperatures, an attempt to secure experimental evidence of this was made. The results of these studies, however, require separate consideration and will be given in detail in another paper. Let it suffice here to note that in twenty-five strains of the pneumococcus, growth was completely inhibited between 40.5° C. and 41.2 C., temperatures often attained or exceeded in the pneumonia patient. When tests of the bacteri-

cidal action of blood sera and exudates were made at temperatures above this, lysis was immediate, marked, and often complete in a few hours. In certain of the most active sera subcultures on plates indicated that lysis was more marked at room temperature than at incubator temperature, for incubator temperature so favored growth that the fleeting lysis was quickly obscured.

In interpreting the significance of these results several factors had to be considered. The most important of these were, (1) simple autolysis of the pneumococcus cell which took place in broth and in salt solution, and (2) inhibition of growth, the latter being apparently the dominant factor.

Summary of the Studies on Bacteriolysis.—It is evident from these experiments that the blood sera of immunized animals vary greatly in their action, but that this variation is one of degree. When sera fail to cause complete lysis no criterion is left by which to measure their activity. In the presence of conditions favoring the growth of the pneumococcus, incomplete lysis permits development of the surviving organisms and whatever action the sera may possess is quickly neutralized. But when by raising or lowering the temperature, growth is prevented, the bacteriolytic action of the sera is marked and often complete. By these means, then, bacteriolysis of the pneumococcus may be demonstrated outside the body in the sera of immunized animals, but how important it is in the tissues during recovery from infection, is still to be determined.

Studies on Phagocytosis.—The destruction of the pneumococcus, however, is not entirely extracellular. Phagocytosis has been recognized and considered an important factor in the recovery of both man and animals. But phagocytosis in pneumococcus infection is apparently an extremely variable phenomenon and previous research has failed to determine the conditions under which it takes place.

Precise determination of the practical significance of phagocytosis has been difficult. Two procedures have been tried. In one of these the leucocytes were examined during infection. In this way the presence of phagocytosis was established directly as in the experiments of Kruse and Pansini (15) and of Tschistovitsch (3).

In the other procedure the opsonic action of the blood sera was studied by Wright's methods, the difficulties of which have already been pointed out in the review of previous work.

Opsonic Action of Immune Sera.—Although technical errors in Wright's methods have robbed the opsonic index of its practical significance at the bedside, under accurately controlled conditions of experiment in the laboratory important qualitative determinations have been made and certain of the quantitative estimates have proved extremely suggestive.

Phagocytosis in Vitro.—In the following experiments the pneumococci were usually grown for twenty-four hours in broth, but occasionally on agar, then centrifugalized and suspended in salt solution. After centrifugalization leucocytes were obtained from the blood of dogs, rabbits, and man, or from the washed pleural exudates of rabbits injected with aleuronat. The mixtures with the sera to be tested were made in equal quantities in the small agglutination tubes. Occasionally the capillary pipettes of Wright were substituted. The bath in which the capillary or other tubes containing the mixtures were immersed was especially designed to insure accurate exposure.[4] The same culture and leucocytes were invariably employed throughout a given experiment. Counts were made of 100 cells, this being adequate for the purposes of the observation. The conditions of experiment thus afford a minimum of error.

It is scarcely necessary to give in detail the long list of my experiments by which it was definitely ascertained that virulent pneumococci are practically insusceptible to phagocytosis. Streptococci and staphylococci were readily taken up, but the pneumococci were not. Typhoid bacilli were taken up and completely digested in ten to twenty minutes, whereas in twenty-four hours the pneumococci had multiplied and none were found to be intracellular.

These facts are now well known and have been established by numerous observers since the beginning of this work. Attempts to secure more active sera failed to alter the result. In some instances agglutination about the leucocytes was noted, and pneumococci

[4] Wadsworth, A., *Proc. N. Y. Path. Soc.*, 1907, vii, 129.

were seen stuck to the leucocytes,[5] but few, if any, were inside the cells.

Hektoen considers insusceptibility to phagocytosis an indication of virulence and suggests the phagocytic index as a measure of virulence. In my experiments with the pneumococcus, virulence was lowered without an increase in susceptibility to phagocytosis, and the phagocytic susceptibility of different strains varied[6] more than their virulence. Thus one species uniformly insusceptible to phagocytosis and of high virulence for the rabbit was allowed to deteriorate during cultivation on media until five cubic centimeters failed to kill a normal rabbit. Up to this point these organisms maintained their insusceptibility. Other strains of the pneumococcus of moderate virulence for rabbits were found which were taken up by the leucocytes.

Streptococci are subject to phagocytosis, and intermediate forms of pneumococci and streptococci doubtless exhibit varying degrees of susceptibility and insusceptibility to phagocytosis just as they do in other characters and reactions. In my experiments it is certain that different strains of pneumococci varied in their insusceptibility to phagocytosis and that this variation was not wholly dependent on virulence as tested on rabbits.

In an attempt to learn something of the nature of this insusceptibility of virulent pneumococci to phagocytosis, the effect upon staphylococcus phagocytosis of the presence of cultures of the pneumococcus was studied.

After twenty-four hours at 37° C. broth cultures of virulent pneumococci taken from a dead rabbit were centrifugalized. The clear culture, and for comparison uninoculated sterile broth, were added to dog leucocytes and incubated for two hours at 37° C., when, after centrifugalizing and decanting, the leucocytes were added to mixtures of the staphylococcus in its immune sera. In ten minutes the leucocytes that had been exposed to sterile broth gave a phagocytic index of 1.44, as compared with 0.76 for the leucocytes exposed to pneumococcus culture fluid.

[5] These were often seen equatorially placed outside the cell limits so that the extracellular position was easily made out.

[6] Atypical pneumococci of little virulence for the rabbit were found later which to some extent were taken up by the leucocytes.

Similarly in another experiment, to three tubes, each containing sediment of pneumococcus broth cultures and dog leucocytes, were added (always in equal quantities), in one instance salt solution, in another normal rabbit serum, and in another the serum of a rabbit immunized to the pneumococcus. After an hour's incubation at 37° C. the leucocytes were obtained by centrifugalizing and decanting the supernatant fluid. The leucocytes from these three tubes were then mixed in equal quantities with staphylococci and staphylococcus immune serum. In ten minutes the leucocytes exposed to pneumococcus sediment and salt solution gave an index of 0.6; those exposed to sediment and normal rabbit serum, 1.5; and those exposed to sediment and pneumococcus immune serum, 2.14.

Again, in still another experiment dog leucocytes were placed in three tubes. To one tube was added a salt solution suspension of a pneumococcus culture from agar and to the second a pneumococcus culture in pneumococcus immune serum. The first two tubes were heated to 37° C. for four hours. The third tube was not heated. Then tubes 1, 2, and 3 were tested with staphylococci and normal rabbit serum, and gave indices of 0.43, 0.96, and 3.08, respectively.

From these experiments it is evident that there are substances in pneumococcus cultures which act on the leucocyte diminishing its phagocytic activity. These substances are present in the culture fluid free from the cells, and in the pneumococcus cells suspended in salt solution. The action of these substances is, in part at least, neutralized by serum of the rabbit immunized to the pneumococcus. If the determination of phagocytic indices were accurate, these experiments would offer an indirect way of measuring the activity of immune sera in neutralizing pneumococcus leucocidins, but the technical error is too great.

Evidence of the toxic action of the pneumococcus growth was also found in the marked degeneration of the leucocytes in the mixtures of pneumococci, leucocytes, and sera. In these the degeneration was less marked when homologous immune sera were present. Later the disintegration of the leucocytes in infected rabbits was noted.

Although immune sera had partially neutralized the toxic action of pneumococcus cultures for leucocytes when these were tested

by staphylococcus phagocytosis, it failed to bring about the phagocytosis of the virulent pneumococci. It was thought that this might be due to growth of the pneumococci in the test mixtures or to the fact that the toxic substances retained in the pneumococcus cell were not neutralized. Accordingly, the study of phagocytosis was conducted at elevated temperatures which not only inhibited growth of the pneumococcus, but, as determined by tests with the staphylococcus phagocytosis, increased materially the activity of the leucocytes. Nevertheless, phagocytosis of virulent pneumococci failed to take place even under these conditions.

Since virulent pneumococci killed by heat or suspended in salt solution were not taken up by the leucocytes, and since elevation of temperature preventing growth failed to bring about phagocytosis, it is evident that the insusceptibility of these pneumococci is due to the retention in the bacterial cell of substances which are to a certain extent thermostabile and not completely neutralized by immune sera.

Under certain conditions in the tissues of infected animals, however, the action of these substances is neutralized and phagocytosis takes places as was early recognized by many other observers in the study of pneumococcus infection.

Phagocytosis in Vivo.—In experiments on pneumonic infection I injected pneumococci of varying grades of virulence through the trachea of normal and immunized rabbits. Varying degrees of lung reaction were incited, but in no stages of the lesions were signs of phagocytosis seen. In the earliest stages pneumococci were present in large numbers, but none were intracellular. In later stages no bacteria were seen. In the animals that recovered, the pneumococci disappeared with astonishing rapidity, suggesting an extremely efficient protective mechanism in the local lesion, adequate in itself, and yet wholly independent of phagocytosis.

The lung lesion, however, marks only a part of the infectious process in pneumonia, and possibly a minor part. The bacteriemia is rarely absent in man. In order to determine the presence or absence of phagocytosis in the bacteriemic processes, the technique had to be modified.

It was found that the leucocytes of the circulating blood might

very easily be examined and that many of the difficulties and errors in the methods of study *in vitro* were here obviated. The blood was drawn from the ear vein into citrate solution and centrifugalized. This was done at low speed to avoid throwing down the pneumococci on the leucocytes.

The leucocytes were pipetted into salt solution, centrifugalized again, and examined in smears for signs of phagocytosis.

To prove that the presence of phagocytosis in the circulating blood could be demonstrated by this method, dense suspensions of the staphylococcus in salt solution were injected into an ear vein of the rabbit. In the blood taken from the other ear ten and twenty minutes later, the staphylococci were found to be intracellular. There were no signs of agglutination present, and the bacteria did not adhere to the leucocytes.

Employing this method for the study of pneumococcus bacteriemia in the rabbit, a large number of the examinations were made at various stages of the infection, early and late, but these revealed no signs of phagocytosis. Moreover, virulent organisms concentrated and suspended in salt solution, or in normal or immune sera, or killed by heat at 52° C. and injected into the ear vein, were found not to be intracellular. Phagocytosis was absent and apparently plays no part in the recovery of the rabbit from pneumococcus infection.

Since Tschistovitsch found phagocytosis in the lung lesions of both dogs and man my studies were repeated on dogs. An active phagocytosis was found in the circulating blood and in the lung lesions, thereby confirming Tschistovitsch's observation, but not necessarily his conclusion, that phagocytosis is the determining factor in the crisis of pneumonia.

Phagocytosis in Man.—Since phagocytosis in pneumococcus infection was shown to vary so markedly in different animals, further study was needed to confirm the claims of other observers and to determine the extent to which it occurs in man. Unfortunately the only available material was that obtained at autopsy.

Twenty-five pneumonic lungs were sectioned, stained for bacteria by Gram's method, and examined for phagocytosis. The results were discouraging. Very few bacteria were found in the exudate,

and there was very little evidence of phagocytosis, and this only in scattered areas. Cases having early lesions, for the most part red hepatization, were selected because previously, in making cultures from gray hepatization, few pneumococci grew. Yet in the earliest case of red hepatization, with death on the third day of the disease, the exudate in the air spaces contained surprisingly few pneumococci. The pneumococci were relatively more numerous in the blood-vessels and it was probably from these that the cultures were obtained.

Bezzola (70) describes the lobulated structure of lobar pneumonia and states that the pneumococci were most numerous in the fresh exudate on the periphery of the lobule and less numerous in the older exudates. Babes (71), Weichselbaum (72), and recently Rosenow (73), note the disappearance of the pneumococci in the exudate. These observers describe it as coincident with fibrinization of the exudate.

Whether these findings are to be attributed to post-mortem changes or to the efficiency of the protective mechanism in the alveolar exudate is not clear. But if they are due to post-mortem changes, it is difficult to understand why the differences in distribution are so constant and why the bacteriemic organisms survive. If in these fatal cases, changes so marked take place, it is difficult to conceive of the protective action of the lung failing to be far more efficient in recovery.

Apart from the study of the material aspirated from the pneumonic lung, practiced under special conditions, there is no way of determining whether the pneumococci are destroyed by phagocytosis or by lysis outside the cells.

Summary of the Studies on Phagocytosis.—It is evident from these experiments that virulent pneumococci are extremely insusceptible to phagocytosis. This is due to the presence of substances which are partly retained by the pneumococcus cell even after it is killed by heat. These substances inhibit the phagocytic activity of leucocytes for the staphylococcus, but are neutralized at least in part by pneumococcus immune serum. Substances set free from the pneumococcus cell are rendered inert by the immune serum; but substances retained by the pneumococcus cell are not acted on

—the virulent organisms, whether living or dead, being in varying degree insusceptible to phagocytosis despite the presence of immune serum. This accounts for the fact that on virulent pneumococci the opsonic action of the blood serum of immunized animals is even less effective than its lytic action.

Non-virulent strains, however, are subject to phagocytosis. On these, immune sera are actively opsonic. But owing to the lack of reliable means of measuring this action, study of the fluctuations in the opsonic index at the bedside of the pneumonia patient have little or no practical value. Study of the phagocytosis of non-virulent pneumococci has thus contributed little to our knowledge of the phagocytosis of virulent organisms in infection, save that, under certain conditions in the tissues, the virulent cells are so altered that they become susceptible, like the non-virulent strains.

In the tissues of some animals, such as the dog and man, phagocytosis is active during recovery; but in others, such as the rabbit, it is absent. Why phagocytosis is absent in one animal, how it is brought about in another, and how important it is, are points still to be determined.

SUMMARY AND CONCLUSIONS.

In summing up the results of these investigations attention is called in particular to the following facts.

Dead pneumococcus culture material does not contain the active poisons formed in infection by living pneumococci. Characteristic lesions are not induced by dead cultures. But substances are present in the pneumococcus cells, and especially in culture filtrates free from pneumococcus cells, that give rise to an immunity in which the poisons of virulent pneumococci are inactive.

In immune sera specific agglutinative, precipitative, lytic, and opsonic activities are present. But to the action of immune sera, virulent pneumococci are singularly insusceptible.

This is due chiefly to qualities acquired by the organisms during their propagation through animals. In the test-tube this insusceptibility is overcome only under exceptional conditions which destroy these qualities or neutralize their effects. Lysis may be brought about by inhibition of growth, and phagocytosis by loss of virulence.

In the tissues inhibition of growth and resistance to the poisons of the pneumococcus are brought about, but in ways more subtle if less exceptional, for both lysis and phagocytosis are active factors in the recovery of certain animals from infection.

BIBLIOGRAPHY.

1. Wadsworth, A., *Am. Jour. Med. Sc.,* 1904, cxxvii, 851.
2. Gamaléia, M. N., *Ann. de l'Inst. Pasteur,* 1888, ii, 440.
3. Tschistovitch, N., *Ann. de l'Inst. Pasteur,* 1890, iv, 285; 1904, xviii, 304.
4. Welch, W. H., *Bull. Johns Hopkins Hosp.,* 1892, iii, 125.
5. Lamar, R. V., and Meltzer, S. J., *Jour. Exper. Med.,* 1912, xv, 133.
6. Fraenkel, A., *Ztschr. f. klin. Med.,* 1886, x, 401; 1886, xi, 437.
7. Talamon, C., *Progrès méd.,* 1883, xi, 281, 301, 1030.
8. Salvioli, G., *Arch. p. le sc. med.,* 1884–5, viii, 127.
9. Foa, P., and Bonome, A., *Ztschr. f. Hyg.,* 1889, v, 424.
10. Foa, P., and Carbone, T., *Riforma med.,* 1891, vii, 127; *Gazz. med. di Torino,* 1891, xlii, 337, 745.
11. Behring, and Nissen, F., *Ztschr. f. Hyg.,* 1890, viii, 412.
12. Bonome, A., *Fortschr. d. Med.,* 1891, ix, 743.
13. Foa, P., and Bordoni-Uffreduzzi, G., *Ztschr. f. Hyg.,* 1888, iv, 67.
14. Emmerich, R., and Fowitsky, A., *München. med. Wchnschr.,* 1891, xxxviii, 554.
15. Kruse, W., and Pansini, S., *Ztschr. f. Hyg.,* 1892, ix, 337.
16. Arkharow, J., *Arch. de méd. expér. et d'anat. path.,* 1892, iv, 498.
17. Klemperer, G. and F., *Berl. klin. Wchnschr.,* 1891, xxviii, 833, 869.
18. Mosny, E., *Arch. de méd. expér. et d'anat. path.,* 1892, iv, 195.
19. Tizzoni, G., and Panichi, L., *Centralbl. f. Bakteriol., Ref.,* 1905, xxxvi, 25.
20. Kruse, W., Allgemeine Microbiologie, Leipzig, 1910, 958.
21. Rosenow, E. C., *Jour. Infect. Dis.,* 1911, ix, 190; 1912, x, 113.
22. Dellezenne, C., and Ledebt, S., *Compt. rend. Acad. d. sc.,* 1911, cliii, 81; *Compt. rend. Soc. de biol.,* 1911, lxxi, 121.
23. Theobald Smith, *Jour. Med. Research,* 1904, viii, 341.
24. Rosenau, M. J., and Anderson, J. F., *U. S. Public Health and Marine Hosp. Serv. Bull.,* 1906–7, Nos. 29, 36.
25. Friedemann, U., *Ztschr. f. Immunitätsforsch., Orig.,* 1909, ii, 591.
26. Friedberger, E., and Goldschmid, E., *Ztschr. f. Immunitätsforsch., Orig.,* 1911, ix, 398.
27. Bail, O., *Arch. f. Hyg.,* 1905, lii, 272; 1905, liii, 302; *Berl. klin. Wchnschr.,* 1907, xliv, 745.
28. Römer, P. H., Experimentelle und klinische Grundslage für die Serumtherapie der Pneumokokkininfektion der menschlichen Cornea (*Ulcus serpens*), Wiesbaden, 1909.
29. Welch, W. H., *Bull. Johns Hopkins Hosp.,* 1890, i, 74.

30. Radziewsky, A., *Ztschr. f. Hyg.,* 1901, xxxvii, 24.
31. Neufeld, F., *Ztschr. f. Hyg.,* 1902, xl, 54.
32. Bordet, J., *Ann., de l'Inst. Pasteur,* 1898, xii, 688.
33. Landsteiner, K., *Centralbl. f. Bakteriol., Orig.,* 1899, xxv, 546.
34. Metchnikoff, E., L'Immunité dans les maladies infectieuses, Paris, 1901, 312.
35. Pettersson, A., *Arch. f. Hyg.,* 1902, xliii, 49.
36. Hoessli, H., *Centralbl. f. Bakteriol., Orig.,* 1910, lv, 135.
37. Dold, H., *Arb. a. d. k. Gsndhtsamte,* 1911, xxxvi, 419.
38. Mennes, F. R., *Ztschr. f. Hyg.,* 1897, xxv, 413.
39. Issaeff, B., *Ann. de l'Inst. Pasteur,* 1893, vii, 260.
40. Denys, J., and Leclef, J., *La Cellule,* 1895, xi, 177.
41. Wright, A. E., and Douglas, S. R., *Proc. Royal Soc.,* London, 1904, lxxii, 357; 1905, lxxiv, 147.
42. Neufeld, F., and Rimpau, W., *Ztschr. f. Hyg.,* 1905, li, 283.
43. Hektoen, L., *Jour. Am. Med. Assn.,* 1911, lvii, 1579.
44. Van de Velde, H., *La Cellule,* 1894, x, 401; *Ann. de l'Inst. Pasteur,* 1896, x, 580.
45. Neisser, M., and Wechsberg, F., *Ztschr. f. Hyg.,* 1901, xxxvi, 299.
46. Bail, O., *Arch. f. Hyg.,* 1898, xxxii, 132.
47. von Lingelsheim, W., *Beitr. f. exper. Therap.,* 1899, No. 1, 1.
48. Rosenow, E. C., *Jour. Infect. Dis.,* 1907, iv, 285.
49. Tschistovitch, N., and Jurevitsch, W., *Centralbl. f. Bakteriol., Ref.,* 1908, xlii, 193.
50. Neufeld, F., and Haendel, *Ztschr. f. Immunitätsforsch., Orig.,* 1909, iii, 159; *Arb. a. d. k. Gsndhtsamte.,* 1910, xxxiv, 166, 293.
51. Rosenow, E. C., *Jour. Infect. Dis.,* 1906, iii, 683.
52. Potter, N. B., and Krumwiede, C., *Jour. Infect. Dis.,* 1907, iv, 601.
53. Seligmann, E., and Klopstock, F., *Ztschr. f. Immunitätsforsch.,* 1909, iv, 103; Seligmann, *idem,* 1909, iii, 159.
54. Boettcher, G., *Deutsch. Arch. f. klin. Med.,* 1909, xcviii, 93.
55. Strouse, S., *Jour. Exper. Med.,* 1909, xi, 743; 1911, xiv, 109.
56. North, C. E., Presentation at the meeting of the Association of American Physicians, June, 1907, unpublished.
57. Park, W. H., *Jour. Am. Med. Assn.,* 1908, l, 639; *N. Y. Med. Jour.,* 1908, lxxxvii, 424.
58. Patella, V., *Atti d. r. Accad. di Roma,* 1888, iv, 447.
59. Buchner, H., *Arch. f. Hyg.,* 1890, x, 84.
60. Pettersson, A., *Centralbl. f. Bakteriol., Orig.,* 1905, xxxix, 423, 613; 1910, liv, 131; 1911, lx, 286; *Ztschr. f. Immunitätsforsch., Orig.,* 1910, vii, 693.
61. Wassermann, M., *Deutsch. med. Wchnschr.,* 1899, xxv, 141.
62. Hiss, *Jour. Med. Research,* 1908, xiv, 323.
63. Flexner, S., *Jour. Exper. Med.,* 1911, xiii, 1.
64. Lamar, R. V., *Jour. Exper. Med.,* 1911, xiii, 6, 380; 1911, xiv, 256.
65. Roux, E., and Yersin, A., *Ann. de l'Inst. Pasteur,* 1888, ii, 629; 1889, iii, 273.
66. Behring and Kitasato, *Deutsch. med. Wchnschr.,* 1890, xvi, 1113.
67. Kitasato, *Deutsch. med. Wchnschr.,* 1889, xv, 635.
68. Prudden, T. M., and Hodenpyl, E., *N. Y. Med. Jour.* 1891, liii, 637; 1891, liv, 617.

69. Wadsworth, A. B., *Jour. Med. Research,* 1903–4, v, 228.
70. Bezzola, D., *Virchows Arch. f. path. Anat.,* 1894, cxxxvi, 345.
71. Babes, V., *Compt. rend. Soc. de biol.,* 1909, lxvii, 725.
72. Weichelsbaum, A., *Med. Jahrb.,* 1886, i, 481.
73. Rosenow, E. C., *Jour. Infect. Dis.,* 1911, viii, 500.

STUDIES ON PNEUMOCOCCUS INFECTION IN ANIMALS.

Second Paper: Action of Immune Sera on Pneumococcus Infection.*

By AUGUSTUS B. WADSWORTH, M.D.

(From the Department of Bacteriology of Columbia University, College of Physicians and Surgeons, New York.)

In my first paper on "Studies on Pneumococcus Infection in Animals" I summarized in the introduction the significant results of previous work and then recorded my experimental studies on the action of the pneumococcus on animal tissues and of immune sera on the pneumococcus. The present paper deals with the action of immune sera on pneumococcus infection and with the mechanism of recovery in animals.

The earliest observers, Foa and Carbone, Emmerich and Fowitsky, Kruse and Pansini, G. and F. Klemperer, and many others mentioned in the introductory summary of the first paper, record experiments showing the effect of immune serum on pneumococcus infections of animals and human beings. G. and F. Klemperer (1) treated inoculated mice with immune serum which they obtained from animals immunized with culture precipitates. In these experiments and in a limited study of the treatment of pneumonia in human beings they found this serum effective and considered its action antitoxic. Although these results were considered favorable by the Klemperers, they have recorded no other experiments. Similarly, on the basis of favorable effects in studies on animals several observers, notably Pane (2), Römer (3), and Neufeld (4), have since urged the use of immune sera in pneumococcus infection of man. But in the hands of others, these sera have failed to yield convincing results. Thus, previous attempts to secure an efficient serum for the treatment of pneumonia in man

* Received for publication, March 28, 1912.

have failed. It is evident, however, from all this study that immune sera have a definite though limited action on pneumococcus infection.

In animals this is manifest under certain conditions of experiment. Immune sera mixed with the virus, or given before or simultaneously with the introduction of the virus, exert marked protective properties. Infection does not develop, or if it does, it is evanescent and not fatal, the result depending on the activity or dosage of the serum and the strength of the virus. Experiments of this kind have given uniformly definite results and form the basis of many of the recommendations for the treatment of man.

When immune sera have been given after infection has become established they have been found to possess little curative action. If treated very early, many animals are saved, but this action is more protective than curative. Occasionally the infection is prolonged and a few animals are saved. The results of experiments of this character are conflicting. Comparatively few observers claim significant practical results, and the great majority acknowledge failure.

In order to determine why immune sera possess marked protective, but slight curative action, the following study was made of the action of immune sera on pneumococcus infection in the rabbit.

STUDIES ON THE CURATIVE ACTION OF IMMUNE SERA ON PNEUMOCOCCUS INFECTION OF THE RABBIT.

From the study of the action of dead pneumococcus cells and of culture filtrates recorded in the first paper, it was found that although this culture material did not contain the active poisons formed by the organisms in infection, substances of a similar or a related nature were present. These gave rise to immunity in animals. In the following experiments the serum of these animals was tested as to its curative value.

Experiments with Antibacterial Sera.—In order to exclude all soluble products of the pneumococcus cell metabolism, it was necessary to use, not only washed pneumococcus cells, but dead cells to prevent the development of soluble products in the body tissues

after inoculation. Broth cultures after growing for twenty-four hours were centrifugalized, the sediment shaken in salt solution, and again centrifugalized. This sediment after being taken up in a small quantity of salt solution made a dense, cloudy suspension, representing a concentration of the culture to one tenth of its original volume or less. To kill the pneumococci these suspensions were exposed in a bath at 52° to 55° C. for twenty minutes.

With this material rabbits were immunized by intravenous inoculation. Two weeks after they had fully recovered from the last inoculation, they were bled and the curative value of the serum was tested by treating rabbits that had been inoculated with virulent pneumococci.

These experiments need not be cited in detail because in none was there definite evidence that this serum possessed greater curative action than normal rabbit serum. In one experiment a few of the treated animals died before the untreated controls.

Experiments with Antitoxic Sera.—In the previous study of dead culture material, filtrates of the cultures incited an immunity that was proportionately greater or more active against virulent infection than that obtained with concentrated cultures or pneumococcus cells in salt solution. Since antibacterial or endotoxic sera failed to prove of any practical value, attention was turned to the study of the antitoxic serum obtained from animals immunized with culture filtrates.

In order to avoid, as far as possible, the liberation into the culture fluid of endotoxins from the pneumococcus cells, twenty-four hour broth cultures were filtered through a Berkefeld filter.[1] These filtrates were tested by subculture in broth to prove their sterility. They were then used for immunization by intravenous inoculation and the curative action of the serum from these animals was tested as in the previous experiments with antibacterial sera.

Many of these experiments, like the previous ones, need not be

[1] The pneumococcus undergoes early autolysis in culture even under the most favorable conditions for growth, but this is not apparent until the growth is on the wane. Estimates of the rate of pneumococcus growth in broth suggest that this point is reached shortly after twenty-four hours, depending on the conditions present. Autolysis is apparent after the second and third days, depending also on the conditions present.

cited in detail for the results failed to show that these antitoxic sera had much practical curative value. The course of the infection in the treated animals, however, was occasionally materially prolonged. There were also signs of improvement in the condition of the animals and occasionally a critical fall in the temperature following the serum treatment. As studied from the records, these signs were not convincing. But as seen in the animals they were striking enough to suggest the presence of antitoxic immunity in pneumococcus infection.

For an instance of this the following experiment is cited.

Experiment 1.—June 6, 1911. Two rabbits were inoculated intravenously with 1.5 c.c. of a culture of pneumococcus R, which was exceedingly virulent, and similarly, two rabbits were inoculated with cultures of pneumococcus H, which was less virulent. One of each set, seemingly the sickest, was chosen for treatment with serum A obtained from an animal highly immunized with culture filtrates of pneumococcus R.

5 c.c. of this serum was given six and a quarter hours after inoculation,—too late to have any material effect on the infections with culture R, but not too late to affect the infections with culture H.

Of the two animals inoculated with the homologous culture pneumococcus R, the control died in eight hours and the treated animal in less than twenty-four hours. Taking the control as a criterion, the serum was given ante mortem.

Of the two animals inoculated with the heterologous culture H, the control died in less than twenty-four hours, but the treated animal lived. The bacteriemia persisted and on the third day the animal was given a second dose of the serum. Signs of infection were slight and the temperature fluctuations evanescent after the first day.

As shown by the blood cultures this antitoxic serum had little or no effect on the bacteriemia.

Other sera of this type were less active or the infections were too virulent to show a curative action.[2] In order to secure more definite experimental evidence of the presence of antitoxic immunity in pneumococcus infection, a study was made of it in its active form.

Animals were partially immunized by two doses of culture filtrates, and for purposes of comparison culture sediment in relatively larger quantities was similarly inoculated into animals. After an interval the immunity of these animals was tested by inoculations of virulent organisms. The experiment is recorded in detail in table I.

[2] Although the curative action of these sera was slight the protective action, when the serum was mixed with the culture before inoculation, was well marked.

Experiment 2.—February 9, 1911. After twenty-four hours' incubation, 300 c.c. of a pneumococcus R broth culture were centrifugalized. The supernatant clear culture fluid was decanted and filtered free of bacteria for immunizing purposes (A). The sediment or concentrated culture was divided in two portions: 5 c.c. was heated to 52° to 55° C. for twenty minutes to kill the bacteria before using them for immunization (B); 10 c.c. was precipitated with 60 c.c. of alcohol and centrifugalized, and the precipitate was partly dissolved in 20 c.c. of distilled water and again centrifugalized. The clear solution (C) and the sediment (D) shaken with water were used. Thus, for the purposes of this experiment, A represents immunization to soluble products; B, concentrated culture killed by heat; C and D, concentrated culture killed and precipitated by alcohol. Thirteen days after immunization the eight animals and two fresh normal animals received intravenously virulent cultures of the pneumococcus R.

In the immunization procedure of this experiment as recorded in table I, material A (culture filtrate) and B (culture sediment heated to 52° C.) failed to cause a toxic reaction in the pneumococcus immunization without living organisms. Material C and D, each derived from culture sediment precipitated with alcohol, gave rise to unusually severe toxic reaction, but without elevation of temperature. The immunization attained by these two inoculations varied in degree and kind. When compared with their controls this immunization was definitely marked in all the animals. The two controls died in less than twenty hours after inoculation. The immunization by culture filtrate differed, being less effective in degree and yet sufficing to neutralize the products of the infectious agents for a time. In one instance this was inadequate, the animal dying in forty-eight hours, but showing modified fever reaction; in the other instance it was adequate, the animal having no fever or signs of infection and ultimately recovering. The infectious agents persisted for nine days as harmless parasites.

The blood of the other animals at this time proved sterile, and it is also interesting to note that although the immunity produced by culture filtrate A failed to prove as effective[3] against the infection, the disease reactions on the whole, as compared with the others, especially with the immunity obtained with culture sediment B, were more completely neutralized. The immunity of C and D for present purposes was quite similar to that of B.

The presence of an antitoxic immunity was thus established. The culture fluid of the pneumococcus after growth for twenty-four hours contains substances capable of inciting an immunity in the tissues which will so neutralize the products of the pneumococci present in the tissues that they give rise to few or no signs of disease.[4] Yet neither with culture filtrates nor with dead pneumo-

[3] In this experiment the animals immunized with culture sediment B, C, and D, received, as above recorded, proportionately greater quantities of material.

[4] This action and reaction between soluble pneumococcus poison and the immunity suggests immediately the toxin and antitoxin reaction of diphtheria. The two are doubtless similar if not identical in nature, but the toxin of diph-

TABLE I.

Experiment 2, Page 82.

		Feb. 9. 3.30 P. M.	Feb. 10. A. M.	Feb. 10. P. M.	Feb. 11. A. M.	Feb. 13–19.	Feb. 23. A. M.	Feb. 23. 11 A. M.	Feb. 23. P. M.	Feb. 24. A. M.	Feb. 24. P. M.
I	Temperature and result						101.8		105.2	Dead	
	Inoculation (intravenous)							0.5 c.c.			
II	Temperature and result						101.4		105.8	Dead	
	Inoculation (intravenous)							0.05 c.c.			
III	Temperature and result	101.2	102	102	101.2	Normal	102		103.2	103.4	104.2
	Immunization (intravenous)	5 c.c. A		5 c.c. A							
	Inoculation (intravenous)							0.5 c.c.			
	Blood culture (1 c.c.)										
IV	Temperature and result	102.4	102.6	102.6	101	Normal	102.5		104.8	102.2	101.8
	Immunization (intravenous)	5 c.c. A		5 c.c. A							
	Inoculation (intravenous)							0.5 c.c.			
	Blood culture (1 c.c.)										
V	Temperature and result	102	102.6	102.8	103	Normal	103		105.4	104.6	104.4
	Immunization (intravenous)	0.5 c.c. B		2 c.c. B							
	Inoculation (intravenous)							0.5 c.c.			
	Blood culture (1 c.c.)										
VI	Temperature and result	102	102	101.8	102	Normal	102		104	105	101.4
	Immunization (intravenous)	0.5 c.c. B		2 c.c. B							
	Inoculation (intravenous)							0.5 c.c.			
	Blood culture (1 c.c.)										
VII	Temperature and result	102.2	101.6	102	102	Normal	101		103	105.8	105.2
	Immunization (intravenous)	2.5 c.c. C		2.5 c.c. C							
	Inoculation (intravenous)							0.5 c.c.			
	Blood culture (1 c.c.)										
VIII[10]	Temperature and result	101.6	102.2	101.8	98.6	Dead, Feb. 19					
	Immunization (intravenous)	2.5 c.c. C		2.5 c.c. C							
	Inoculation (intravenous)										
	Blood culture (1 c.c.)										
IX	Temperature and result	101.6	102.4	102	101.2	Normal	101		103.4	104.6	104
	Immunization (intravenous)	2.5 c.c. D		2.5 c.c. D							
	Inoculation (intravenous)							0.5 c.c.			
	Blood culture (1 c.c.)										
X[10]	Temperature and result	102.4	101	101.2	100	Dead, Feb. 13					
	Immunization (intravenous)	2.5 c.c. D		2.5 c.c. D							
	Inoculation (intravenous)										
	Blood culture (1 c.c.)										

[10] Animal VIII was sick after inoculation and there was a marked fall in temperature. Later diarrhea de
died. At autopsy the intestines were anemic, and the blood-vessels were injected with blood, but smears
from bacteria. Animal X died also, and at autopsy smears and cultures failed to show bacteria, although th
increased and blood-tinged, as it often is in active infections.

Feb. 24.		Feb. 25.		Feb. 26–28.	Mar. 3.
A. M.	P. M.	A. M.	P. M.		
ead					
ead					
3.4	104.2	Dead			
2.2	101.8	102	102.8	Normal	102.8
					13
4.6	104.4	104	103	Normal	103
					0
5	101.4	104	105.4	Normal	102
					0
5.8	105.2	102.4	103.4	Normal	101
					0
4.6	104	102.6	103	Normal	102
					0

arrhea developed and the animal
smears and cultures were free
though the pericardial fluid was

Exper

		June 13.			
		1.30 P. M.	5.30 P. M.	8 A. M.	A.
I	Temperature and result	101	105	Dead	at
	Intravenous inoculation	0.05 c.c.		18 hou	
	Untreated				
II	Temperature and result	102.2	103.6	103.6	De
	Intravenous inoculation	0.05 c.c.			2
	Untreated				
III	Temperature and result	102.4	104.4	105.4	
	Intravenous inoculation	0.05 c.c.			
	Treatment (intravenous)				
	Normal serum		1.0 c.c.		1.0
IV	Temperature and result	102.2	104.6	106	
	Intravenous inoculation	0.05 c.c.			
	Treatment (intravenous)				
	Normal serum		1.0 c.c.		1.0
V	Temperature and result	101	104	105	
	Intravenous inoculation	0.05 c.c.			
	Treatment (intravenous)				
	Immune serum		1.0 c.c.		1.0
VI	Temperature and result	102.8	105	104.4	
	Intravenous inoculation	0.05 c.c.			
	Treatment (intravenous)				
	Immune serum		1.0 c.c.		1.0
VII	Temperature and result	102.2	104.8	105	
	Intravenous inoculation	0.05 c.c.			
	Treatment (intravenous)				
	Immune serum		1.0 c.c.		1.0
VIII	Temperature and result	102.8	104.8	104	
	Intravenous inoculation	0.05 c.c.			
	Treatment (intravenous)				
	Immune serum		1.0 c.c.		1.
IX	Temperature and result	101.1	105.8	106	
	Intravenous inoculation	0.05 c.c.			
	Treatment (intravenous)				
	Immune serum		1.0 c.c.		1.
X	Temperature and result	102.4	104.4	105	
	Intravenous inoculation	0.05 c.c.			
	Treatment (intravenous)				
	Immune serum		1.0 c.c.		1.

ABLE II.

ment 3, Page 83.

June 14.				June 15.		June 16.		June 17.	June 18.	June 20.	June 22.
M.	1.30 P. M.	5 P. M.	6.30 P. M.	A. M.	P. M.	A. M.	P. M.	A. M.			
er ..											
d after hours.											
	105.4	106		105.2	Dead after 48 hours.						
c.c.			1.0 c.c.								
	105.8	105.2		105	Dead after 48 hours.						
c.c.			1.0 c.c.								
	104	104.2		102.4	102.6	101.6	102	102.8			Normal.
c.c.			1.0 c.c.								
	103.2	104.2		103.4	103.6	102	101	102.8		Dead	
c.c.			1.0 c.c.								
	103.2	103.2		104.2	105.8	103.6	103.4	101.8	Dead		
c.c.			1.0 c.c.								
	103	104.2		102.8	103.6	103.8	102	103.8			Normal.
c.c.			1.0 c.c.								
	104	104.8		104.2	103.2	104.6	104.6	104.2			Normal.
c.c.			1.0 c.c.								
	102.5	103		104.2	105	104	104.6	102			Normal.
c.c.			1.0 c.c.								

TABLE III.

Experiment 4, Page 84.

		June 10.							June 11.	June 12.	
		10 A. M.	11.30 A. M.	12 M.	12.30 P. M.	2.30 P. M.	5 P. M.	6.30 P. M.		A. M.	P. M.
I	Temperature and result	102				105	105.4		Dead in 20 hours.		
	Intravenous inoculation		0.25 c.c.								
	Untreated										
II	Temperature and result	101.8				104.6	105.4		Dead in 20 hours.		
	Intravenous inoculation		0.25 c.c.								
	Untreated										
III	Temperature and result	102				105	105		105.4	Dead after 43 hours.	
	Intravenous inoculation		0.25 c.c.								
	Treatment (intravenous)										
	Immune serum							2.0 c.c.			
IV	Temperature and result	101.6				105.2	106		105.6	Dead after 44 hours.	
	Intravenous inoculation		0.25 c.c.								
	Treatment (intravenous)										
	Immune serum							2.0 c.c.			
V	Temperature and result	101.2				104.2	106		104.6	104.4	104
	Intravenous inoculation		0.25 c.c.								
	Treatment (intravenous)										
	Immune serum					2.0 c.c.					
VI	Temperature and result	102.4				104.6	104.8		104.4	104.8	103.
	Intravenous inoculation		0.25 c.c.								
	Treatment (intravenous)										
	Immune serum					2.0 c.c.					
VII	Temperature and result	102				105.2	105.2		103	104.4	103.
	Intravenous inoculation		0.25 c.c.								
	Treatment (intravenous)										
	Immune serum				2.0 c.c.						
VIII	Temperature and result	102				105	104.6		106	105.2	105.
	Intravenous inoculation		0.25 c.c.								
	Treatment (intravenous)										
	Immune serum				2.0 c.c.						
IX	Temperature and result	102				104.6	104.4		104	104	102
	Intravenous inoculation		0.25 c.c.								
	Treatment (intravenous)										
	Immune serum			2.0 c.c.							
X	Temperature and result	102.2				104.6	103.6		104	103	104
	Intravenous inoculation		0.25 c.c.								
	Treatment (intravenous)										
	Immune serum			2.0 c.c.							

June 13.		June 14.		June 22.
A. M.	P. M.	A. M.	P. M.	
104.4	104.4	103.4	103.8	Normal.
101	Dead after 72 hrs.			
105.6	103.6	103	103.4	Normal.
105.4	103.6	104.2	104	Normal.
103.2	104	103.8	103.4	Died, but not of pneumococcus infection.
104.2	104	104.2	105.4	Normal.

coccus cells could this immunity be sufficiently exalted to yield sera of any definite practical value in the treatment of infection in animals.

In cultures the pneumococcus does not develop the very active poisons it forms in infection; hence my study turned to that of sera derived from immunization with living cultures.

Experiments with Sera Obtained by Immunization with Living Cultures.—Since the intravenous inoculation of culture filtrates had proved so effective in immunization, rabbits were first thoroughly immunized with these; then, in increasing doses, the living virulent cultures were substituted until the animals withstood the inoculation of large quantities without giving any signs of reaction. The blood serum was tested as in the previous experiments with antitoxic and antibacterial sera.

For convenience and to show in detail the effect of these sera the results of certain typical sets of experiments are tabulated. In table II (experiment 3) is shown the effect of repeated treatment. Three one cubic centimeter doses of immune serum and three of normal serum were administered at intervals during the first twenty-nine hours of the infection. In table III (experiment 4) is shown the effect of a single dose of two cubic centimeters given to different animals at varying intervals after the inoculation of five times the quantity of virus used in table II. And table IV shows the curative action of this serum on homologous and heterologous infections.

Experiment 3.—June 13, 1911. Of ten normal rabbits, each received 0.05 c.c. of an extremely virulent culture of pneumococcus R. Two rabbits were untreated and two were treated with normal rabbit serum. These four animals served for control. The other six were treated with serum B (from rabbits highly immunized with living cultures).

In table II the virulence of the organisms is well shown in the two controls which died eighteen and twenty-one hours respectively after inoculation. The slight but inadequate protective value of normal sera is also well shown by the death in forty-eight hours of each of the two treated animals.

theria, although unknown chemically, is a powerful poison that is fatal for animals, and this poison may be titrated accurately against its antitoxin, whereas the soluble substances of the pneumococcus are not fatal and are recognized only by the immunity incited by their presence. Moreover the immune products can be measured only by their activity against infection.

The curative value of the immune serum is clearly demonstrated by the complete recovery of four of the six animals receiving this treatment. The two that died lived five and seven days, i. e., two and a half, and three and a half times as long as the controls similarly treated with normal serum. Although this serum was administered in these small doses, it never failed to produce marked betterment in the animals' condition, and the temperatures taken after the serum treatment are lower than those in the animals treated with normal serum.

It is therefore evident that both normal rabbit sera and immune sera exert a beneficial action when used in the treatment of infected animals. The effect of the serum from the normal rabbit is slight and evanescent. But in the serum from the rabbit highly immunized with living cultures this action may be greatly exalted, and may modify and prolong the course of fatal infections, or speedily effect permanent cures.

In order to determine the effectiveness of a single dose of serum administered at varying intervals after infection was established, in the following experiment (table III) were employed double the dose (two cubic centimeters) of immune serum, and five times as much of the culture for inoculation, as was used in table II.

Experiment 4.—June 10, 1911. Ten rabbits were inoculated with 0.25 c.c. of this virulent culture of pneumococcus R, and subsequently at intervals of a half hour, one hour, three hours, and seven hours; all but two of these rabbits received 2 c.c. injections of serum B obtained from rabbits which had been highly immunized with living cultures of pneumococcus R.

In table III the results of this experiment show very clearly the effect of 2 c.c. doses of immune serum, each given at varying intervals after the virulent inoculation of 0.25 c.c. of pneumococcus culture. The fall in temperature of the treated animals was often marked, and strikingly uniform. The controls died in less than twenty hours. The animals treated seven hours after inoculation died in forty-three and forty-four hours. Of those treated three hours after inoculation one lived and one died in seventy-two hours. All those treated half an hour and one hour after inoculation recovered.

Under the conditions recorded in table III, and with the same quantity of virus and the same dosage of serum it is evident that three hours after the injection of the virus is the limit of delay in treatment which will allow of a cure by a single dose of the serum.

If serum treatment is to be effective, it must be given within a certain interval after the inoculation of virulent organisms; after this interval has elapsed, treatment is ineffective. This is true alike in infection and in toxemia without infection.

Soon after the toxins of diphtheria and tetanus are injected, they are so fixed by the animal cells, or the animal cells are so in-

jured, that recovery is no longer possible and antitoxin fails to cure. In the toxemia caused by the tetanus and diphtheria toxins, the time required for the fixation of the poison or for the injury to the cells is definite, but depends within certain limits on the quantity of toxin injected and on the susceptibility of the animal tissues to the toxin. In generalized pneumococcus infection of the rabbit, the development of the bacteria and their virulence complicate still further the conditions present in toxemia without infection.

In order to determine the effect on homologous and heterologous infections of serum from animals immunized with living pneumococci the following experiments were carried out with the homologous organism R and the heterologous organism H.

Experiment 5.—Four animals were inoculated intravenously with 0.5 of a cubic centimeter of an extremely virulent culture of R, and three with a less virulent culture of H. Immune sera derived from immunization with living cultures of R were given intravenously three hours after inoculation, each dose being one cubic centimeter. The records of this experiment appear in table IV.

In experiment R, recorded in table IV the controls died in less than eighteen hours. Of the treated animals one died on the third day, the other recovered. Against 0.5 c.c. of this virulent homologous culture a single dose of 1 c.c. of this serum given three hours after inoculation cured one animal and caused the infection in the other to be nearly four times as long as that in the control.

In experiment H the control died on the fifth day. Against 0.5 c.c. of this heterologous culture a single dose of 1 c.c. of this serum given three hours after inoculation induced crisis and effected a complete cure in both animals. Animal III died from an accident. At autopsy, despite extensive hemorrhage into the muscles of the neck, no pneumococci were present.

It is evident from these experiments that immune serum obtained by immunization with living cultures may be, like the antitoxic serum (see page 81), active on both heterologous and homologous infections, but the action here is more marked than that of the antitoxic serum.

SUMMARY OF THE STUDIES ON THE CURATIVE ACTION OF IMMUNE SERA ON PNEUMOCOCCUS INFECTION.

In one set of experiments pneumococcus cells washed free of soluble products were killed by heat at 52° C. and injected into animals. In another set of experiments culture filtrates freed of pneumococcus cells that had been growing for twenty-four hours

were employed. Both sets of experiments gave rise to an immunity in the body tissues. This immunity in the tissues was well marked in each instance, but in certain respects the immunity produced by the injection of the dead cells differed from that induced by the injection of the cell-free filtrates. After immunization with culture filtrates the tissues acquired an immunity which was distinctly selective and antitoxic, for in animals immunized in this way the toxic products of the pneumococci were neutralized and the surviving parasites became harmless. On the other hand, after immunization with pneumococcus cells, the tissues acquired an immunity which was effective in its active form but one which was stronger in its bactericidal action on the pneumococci in infection and weaker in its antitoxic action than the immunity induced by the culture filtrates. Nevertheless, as the pneumococcus cell contains some of the substances present in the culture filtrates, the immunity produced by dead cells may be, to a certain degree, antitoxic.

The immunity to the pneumococcus cells and to the culture filtrates was thus readily studied in its active form, but it was never sufficiently exalted by immunization with this culture material to yield potent sera. The antibacterial sera had little or no curative action, the antitoxic sera proved only slightly better; and neither gave promise of practical usefulness.

The serum obtained with living virulent cultures, however, proved by contrast definitely curative. Although no attempt was made in these experiments to measure the extreme limits of its curative action, the results leave no doubt of its activity against both homologous and heterologous infections.

These sera varied greatly in their curative action: some proved efficient, others of little value. With improved methods of immunization with pneumococcus R this variation was less marked. Other strains of the pneumococcus were apparently less serviceable. Whether this was due to differences in the methods used or to differences in the strains was not clearly determined.

It was thought that certain strains of the pneumococcus were more toxic than others. Certainly some virulent organisms gave rise to extensive hemorrhagic lesions not present in other fatal infections. The organism R, used so frequently in these experi-

ments, was of this toxic type. But, important as it is to solve these questions, they are scarcely within the scope of this paper.

Having clearly demonstrated the limitations in the usefulness of certain immune sera and the marked efficiency of others, an attempt was made to determine how these effected cure.

In the recovery from infection the protective mechanism acts in two ways. It neutralizes the products of the infectious agents, and destroys the bacterial cells. The toxins, the endotoxins, the aggressins and the leucocidins are neutralized in the specific reactions of immunity, and the bacterial cells are destroyed by lysis extracellularly or by phagocytosis intracellularly.

How much is to be gained by the present complex conceptions of the protective activities is not yet clear, but they have already contributed much valuable information to our knowledge of the manner in which the adaptive reactions of immunity take place. Nevertheless, how fundamental all these distinctions are, or how they will hold in the light of complete knowledge, is still to be determined. Although extremely important and of great theoretical interest, these finer distinctions of the minor, or secondary, phases of the protective mechanism have not only failed to yield practical results in the cure of disease, but have often proved misleading, and particularly so in the attempts to cure pneumonia in man.

The simpler conceptions of the early observers, now so long discarded, are far more practical. In these, toxin and antitoxin neutralization are dominant, but a convincing experimental demonstration of their presence has been lacking. And yet it is difficult to interpret immunity to culture filtrates in any other way. In the filtrates of cultures that have grown for twenty-four hours substances are present which give rise to immunity. This fact renders scarcely tenable the classification of these substances as endotoxin, as aggressin, or as antigen for the development of lytic or opsonic activities. It would be far more reasonable to classify in this way diphtheria toxin which is obtained from cultures after several days' growth. Failure of these substances to prove as markedly toxic as diphtheria or tetanus toxin does not exclude their being of a related or similar nature. Diphtheria toxin may be so altered through degen-

eration or under experimental conditions that it no longer is actively toxic, but in the tissues it is still capable of producing antitoxin which will neutralize active toxin. Similarly non-virulent strains of the diphtheria bacillus give rise to substances not actively toxic but capable of producing antitoxin. In fact it is by these reactions that non-virulent and non-toxic strains are identified.

But in my experiments thus far recorded, toxin and antitoxin reactions were clearly recognized only in the active phases of immunity. In passive phases the evidence was suggestive, but unconvincing and of no practical moment. It was only with serum obtained by immunization with the living pneumococci that the protective mechanism was manifested experimentally in passive phases. With serum derived from living organisms as compared with that obtained by culture filtrates the reactions are complex. In these complex reactions, factors other than toxin and antitoxin neutralization might well be conceived as active or even dominant. In order to determine this the mechanism of recovery was studied.

In studies of lysis and phagocytosis recorded in the first paper, phagocytosis was practically absent in the rabbit. Typical pneumonic lesions do not develop in the rabbit, whereas they are readily induced experimentally in the dog. Accordingly, in seeking light on what actually takes place in the pneumonia of man, I studied the mechanism of recovery in the dog instead of in the rabbit.

STUDIES ON THE MECHANISM OF RECOVERY IN THE DOG.

In an unpublished study, made by the author in 1905, of the conditions underlying the development of organizing pneumonia, extensive lesions of lobar pneumonia were readily induced by inserting a catheter into the trachea of etherized dogs and injecting virulent cultures of pneumococci through it to any desired part of the lung. This method was therefore adopted in these experiments on dogs.

Experiments on Lung Infection of the Dog.—On February 20, 1911, two dogs were etherized and 30 c.c. of a culture of pneumococcus R from a dead rabbit were injected through the trachea. Dog 1 was not sick the first day after the injection, but became sick on the second day, and on the third day had a temperature of 104.6° F. Dog 2 was very sick at first with a temperature of 104.5° F., but made a quick recovery and was practically normal on the third day after the injection, when both dogs were pithed and autopsied.

In dog 1 extensive lesions of hepatization in the lower right lobe were found; in dog 2, circumscribed areas of consolidation. The heart blood, and the exudates squeezed from the pneumonic lesions and from the pleura, were centrifugalized in citrate solution, the leucocytes pipetted off, again centrifugalized in salt solution, and the sediment smeared and examined by Jenner's stain for evidences of phagocytosis. No pneumococci were found inside or outside the leucocytes.

In cultures of the blood and of the lung exudate, pneumococci grew in small numbers, but plates made of small amounts of blood and exudate were sterile. The results of the study in the two dogs were similar despite the different stages of the infection. In dog 1 there were extensive lesions of red hepatization, in dog 2 circumscribed lesions.

The efficiency of the dog's protective processes was thus clearly demonstrated, but no clew was left to show the mechanism by which the infectious agents had been destroyed.

On March 15, 1911, two dogs were etherized, and 35 c.c. of broth culture from a rabbit dying from pneumococcus R infection were injected through the trachea. These dogs were prostrated, but recovered slightly by the third day, when one was treated intravenously with 15 c.c. of immune serum from an immunized dog, and two hours later both were pithed, autopsied, and studied as in the previous experiment. No signs of phagocytosis and in fact no bacteria were seen in the smears of leucocytes centrifugalized from the blood or in the exudates from either animal. In cultures made from the blood and exudate of the untreated animal, one pneumococcus colony was found in 2 c.c. of blood and 344 colonies in 1 c.c. of the exudate. This animal's right upper lobe was completely hepatized and was in the early red stage. In the other animal before treatment two pneumococci were present in 2 c.c of blood, but two hours after treatment the blood culture of this dog was sterile. The lung exudate also was sterile. In this second animal, the right lower lobe was completely hepatized, and was similar to that of the first animal. Whether the results in this dog are to be attributed to the serum treatment or to a more efficient protective mechanism is not apparent.

In this, as in the previous experiment, the protective mechanism of the dog was efficient even in these extensive early lesions and by the third day evidences of the means by which the infectious agents were eliminated had practically disappeared. The quantity of culture inoculated was, therefore, increased and the study was made at the end of twenty-four hours instead of seventy-two hours.

On March 24, 1911, three dogs were etherized, and the tracheal injections of pneumococcus R culture were made proportional to the weight of the dogs, 50 c.c. being injected for each 10 kilos. One dog was untreated. Each of the other two received intravenously 18 c.c. of the serum from a highly immunized goat. In these dogs the temperatures rose to 104° F. and 105° F. In the two that were sickest, physical signs of pneumonia were easily made out, but in the other dog these were doubtful and it was accordingly selected as the control. All three dogs were pithed and autopsied twenty-four hours after inoculation.

In the untreated animal there was a small area of pneumonic consolidation in the lower right lobe. Cultures from the heart's blood and from the exudate were sterile. Although this exudate was sterile, by morphological study a few pneumococci were found inside the leucocytes. Both of the treated animals had extensive lesions of red hepatization in the right and left lower lobes. The blood of one was sterile before the serum treatment and also two hours after it. In the blood of the other, six colonies per cubic centimeter were found before treatment, and twelve colonies two hours after treatment. No signs of phagocytosis were seen in the leucocytes of the blood, but in the lung exudate of both animals signs of phagocytosis were marked, and in culture innumerable colonies grew.

Again in this experiment the action of the serum, which in other tests both in rabbits and in dogs had proved efficient, was not clearly shown. But the protective mechanism and the activity of phagocytes was clearly demonstrated. Evidently the protective mechanism is so efficient and yet so variable that even under these experimental conditions, it is almost as difficult to measure the action of the serum in the recovery of the dog as it is in that of man.

Previous experience with experimental pneumonia in more than seventy-five dogs which were used in other studies on organizing pneumonia, had shown that, apart from the fact that recovery in dogs is so frequent, practically the rule, neither recovery nor death could be definitely foretold.

Meltzer and Lamar (5) note a mortality of 16 per cent. in their series of fifty dogs. It occurred to me that if the pneumonic infection of the dog were taken practically ante mortem the effect of serum treatment might be more apparent.

In two series of my experiments, each consisting of ten dogs, the animals were inoculated through the trachea and their temperatures were carefully watched. After an evanescent rise to 104° F. or 105° F. on the day following the inoculation the temperatures of the animals were practically normal. Although many were extremely sick, all but two recovered. This was attributed to lack of virulence for the dog in the cultures used.

This pneumococcus R had been kept in rabbits by continuous passage. A strain was accordingly passed through four dogs to exalt its virulence for this animal. But virulence is apparently not so readily increased in the dog as it is in the rabbit. Twenty-five

cubic centimeters inoculated intravenously gave rise to an infection that was fatal in three weeks, although in the dogs from which this culture had been isolated the same dose had killed in forty-eight hours. Dogs evidently vary in susceptibility far more than rabbits. The effort is now being made to exalt the virulence of pneumococcus R for dogs to such an extent that it will invariably prove fatal in these animals, but in this attempt I have not yet succeeded.

Experiments on Bacteriemic Infection of the Dog.—Since it was not possible with the cultures at hand to secure uniformly fatal lung infection of the dog a study was made of the bacteriemia after large doses of concentrated culture material.

I. On April 10, 1911, a normal dog received intravenously 10 c.c. of a dense suspension of centrifugalized pneumococcus culture. Before the inoculation of the pneumococci, the blood was sterile and the leucocytes free from phagocytosis. Twenty minutes later in 1 c.c. of blood 68,400 pneumococcus colonies were found, and in the leucocytes, definite signs of a moderately active phagocytosis. Fifty minutes after inoculation, the blood contained 6,000 pneumococci per cubic centimeter and signs of a more active phagocytosis were present in the smears of the leucocyte cream. In about forty hours the animal died of the infection. In the blood at autopsy very few pneumococci and no signs of phagocytosis were found. The leucocytes were comparatively few in number and in many of them signs of degeneration were marked.

II. On the 15th of March, 1911, a normal dog was etherized, the jugular vein was exposed and clamped, and into it in the direction toward the heart was inoculated 5 c.c. of a dense suspension of virulent pneumococcus cells obtained by centrifugalizing 250 c.c. of broth culture. Thirty minutes later blood was drawn from a place in the vein that was well above the site of inoculation. Part of this blood was diluted and plated. It contained 51,670 pneumococci in 1 c.c. The remainder was centrifugalized in citrate solution, the cream pipetted off, centrifugalized again, and then examined in smears for phagocytosis. No free bacteria were seen and very few were found inside of the leucocytes, none of which contained more than two cocci. Signs of phagocytosis were extremely scarce at this stage. Two and a half hours after inoculation the examination of the circulating blood was repeated. There were only 384 pneumococci per cubic centimeter, and relatively few signs of phagocytosis despite the enormous number of pneumococci that had been disposed of.

Evidently the activity of phagocytosis in normal dogs varies considerably, for in this animal, as compared with the previous one, the difference was marked. These, however, represent the extremes of the results of experimentation on the unimmunized dog.

On March 16, 1911, the day following the inoculation of the virus, this second dog was greatly prostrated, and although there was no fever, it was expected to die. On the third day, 35 c.c. of the serum of an immunized dog was injected intravenously. In the blood before the serum treatment nineteen pneumococci were found per cubic centimeter, and after treatment, four. No evidence of phagocytosis was seen, but this might have been due to the small number of bacteria present. Following this treatment, however, the animal recovered rapidly and five days later was again the subject of an experiment.

On March 23, the pneumococci from 250 c.c. of broth culture suspended in 7 c.c. were inoculated intravenously into the dog used in the last experiment, and twenty-five minutes later the animal received intravenously 35 c.c. of immune goat serum. This serum caused slight hemolysis. Before the inoculation of the virus the blood was sterile. Fifteen minutes after inoculation and ten minutes before the serum treatment, 1 c.c. contained 436,296 pneumococci. Phagocytosis was marked. An hour after inoculation and a half hour after the serum treatment 1 c.c. of blood contained only 32 pneumococci, but phagocytosis was if anything still more marked.

On March 13, 1912, a year later, this animal was again studied, after having been left untreated for ten months. The pneumococci (R) in 200 c.c. of broth culture were concentrated, suspended in 20 c.c. of the culture fluid, and inoculated intravenously. One and a half hours after inoculation, blood was drawn from another vein. In a plate containing 1 c.c. of blood, 5,088 colonies of pneumococci were found, and in a plate containing 0.03 c.c. of blood, 3,600 per cubic centimeter.[5] No signs of phagocytosis were seen in the leucocytes obtained by centrifugalizing 2.5 c.c. of blood in citrate solution. These leucocytes, however, gave evidence of the extent to which the animal was poisoned. Many stained faintly, many others appeared as shadow forms, and a few were granular and disintegrating. In short, the signs were those of the toxemia so frequently noted ante mortem in both dog and rabbit, or seen when these animals are seriously poisoned. This dog after inoculation was nauseated and greatly prostrated, but recovered.

By studying this one animal, first in the unimmunized state when phagocytosis was slight, then after an active immunization when phagocytosis was marked, and again a year later when, in the latent stage, phagocytosis was absent, not only is the relationship of phagocytosis to the immunity apparent, but it is evident that the immunity must be in an active state to prove effective in bringing about phagocytosis. When even the immunized dog is badly poisoned, phagocytosis is in abeyance, and the destruction of the pneumococci is then extracellular.

[5] This is an excellent illustration of how variable the growth of the pneumococcus is in plates of agar. The two plates were inoculated and poured under identical conditions. The presence of the blood determined the difference in the results; in the plate containing 0.03 c.c. of blood, many of the pneumococci failed to develop.

A bull terrier that had been well immunized on March 8, 1911, was etherized, 400 c.c. of blood were taken, and the jugular vein was tied. In the stump toward the heart 22 c.c. of a dense suspension of the pneumococci centrifugalized from 500 c.c. of broth were injected. In the blood drawn fifteen minutes later, 385,416 pneumococci per cubic centimeter were found and there was an active phagocytosis. No signs of degeneration were seen in the leucocytes and nearly all contained bacteria. Some leucocytes enclosed as many as 50 pneumococci, and 71 leucocytes taken at random gave a phagocytic index of 5.[6] The dog died in forty hours from the combined effects of bleeding and inoculation.

At autopsy the blood contained 230 pneumococci per cubic centimeter. Signs of degeneration were marked in the leucocytes, and the number of these cells was greatly diminished. In one leucocyte the shadow form of a diplococcus was found. With this exception no signs of phagocytosis were seen in the smears.

In this immunized animal phagocytosis was at first extremely active. Then, as the toxemia developed, phagocytosis disappeared and was absent ante mortem. Even after this overwhelming infection the bacteriemia at autopsy was slight compared with that obtained in the rabbit.

SUMMARY OF THE STUDIES ON THE MECHANISM OF RECOVERY IN THE DOG.

These experiments demonstrate the importance of phagocytosis as a means of disposing of the pneumococci in the infection of the dog. Both in the lesions of the lung and in the blood phagocytosis plays an active part in the mechanism of recovery. The difference in the activity of phagocytosis in the normal dog and in the immunized animal is striking. In the normal dog phagocytosis may be practically absent and in this respect the dog may resemble the rabbit. But phagocytosis is greatly augmented in the actively, and also in the passively immunized animal. Ante mortem, phagocytosis is absent in both normal and immunized animals. It is also absent in the highly immunized animal when the immunity has long been dormant and the unneutralized poisons act on the body cells.

Thus it is evident that phagocytosis in the dog depends very largely upon the extent to which the toxic products of the pneumo-

[6] If there are 10,000 leucocytes to 1 c.mm. of blood and each takes up as few as five bacteria, 500,000,000 per c.c. are disposed of in this way. In the blood culture fifteen minutes after the injection, 385,416 pneumococci were found in 1 c.c. of blood.

coccus are neutralized. Hence it is that although phagocytosis may be a dominant process in the disposal of the pneumococcus cells it is secondary to the specific reactions which take place between the protective substances of the body and the poisons of the bacteria.

As a means of studying phagocytosis in pneumococcus infection, these experiments were especially successful, but in many other respects the results were unsatisfactory. The dog's protective mechanism was so extremely efficient that demonstrations of the action of immune sera were not convincing. Conditions present before treatment with sera and those present after, were not sharply contrasted in the dog. The differences noted were those of degree only and were not so definite as they were in the rabbit. After infection became established the bacteriemia was never so marked as in the rabbit. On the contrary, following the injection of the culture, the pneumococci were invariably destroyed in large numbers and relatively few survived in either treated or untreated animals. The serum simply hastened this destruction of the pneumococci. Even ante mortem the organisms were not numerous. Although the dog developed typical lung lesions, there was no systemic reaction with sustained temperature and no crisis. This was true also of the bacteriemic infection of the dog.

Resistant animals, such as the dog and man, react locally alike, but differ in their systemic reactions. In these reactions man and the rabbit resemble each other more closely.

STUDIES ON THE MECHANISM OF RECOVERY IN THE RABBIT.

In order to study in detail the effect of more active curative sera it was necessary to confine the experiment to a single animal. To control the results, however, the animals were taken in series. In these the control and the test animals alternated. Each rabbit was inoculated with cultures isolated from the one preceding it. An excessive inoculation, five cubic centimeters in each instance, was purposely used to secure exaggerated conditions of bacteriemia and toxemia. There were three controls, and three animals treated with immune sera.

Of the controls, one was left untreated, another was treated with

TABLE IV.

Experiment 5 (R), Page 85.

		April 24.				April 25.				April 26.		April
		1 P. M.	4 P. M.	4.10 P. M.	6 P. M.	9 A. M.	10 A. M.	12 M.	6 P. M.	9 A. M.	6 P. M.	9 A. M.
I	Temperature and result	102.2	105		105.2	Dead						
	Intravenous inoculation (R)	0.25 c.c.										
II	Temperature and result	102.6	105.2		105.8	Dead						
	Intravenous inoculation (R)	0.25 c.c.										
III	Temperature and result	102.6	105.2		105.4	104.6	103.8	104.6	104.2	105.8	103.6	102.4
	Intravenous inoculation (R)	0.25 c.c.										
	Treatment (intravenous)											
	Immune serum			1.0 c.c.								
IV	Temperature and result	102.6	105		106	105	104	105.3	104.8			Dead
	Intravenous inoculation (R)	0.25 c.c.										
	Treatment (intravenous)											
	Immune serum			1.0 c.c.								

Experiment 5 (H), Page 85.

		April 28.		April 29.		April 30.		May 1.	May 2.
		6.45 P. M.	10 P. M.	9 A. M.	6 P. M.	9 A. M.	6 P. M.	10 A. M.	10 A. M.
I	Temperature and result	101.6	104.7	105	105.2	105.2	104.4	103.8	101.4
	Pneumococcus H (intravenous)	0.25 c.c.							
II	Temperature and result	102.9	104.6	101.4	100.2	103	102.4	101.5	101.4
	Inoculation pneumococcus H (intravenous)	0.25 c.c.							
	Immune serum (pneumococcus R)		1.0 c.c.						
III	Temperature and result	102.6	105.2	102	102.6	102.8	101.4	102.4	102.2
	Inoculation pneumococcus H (intravenous)	0.25 c.c.							
	Immune serum (pneumococcus R)		1.0 c.c.						

		April 26.		April 27.		April 28.	
		9 A. M.	6 P. M.	9 A. M.	5 30 P. M.	9 A. M.	6 P. M.
		105.8	103.6	102.4	102.8	104.8	104.2. Cured.
				Dead			

		30.	May 1.	May 2.	May 3.	May 4.
		6 P. M.	10 A. M.	10 A. M.	A. M.	A. M.
		104.4	103.8	101.4	Dead	
		102.4	101.5	101.4	101.2	100.8. Cured. Normal thereafter.
		101.4	102.4	102.2	101.6	Cured, but killed by accident. Cultures sterile.

		11.30 A. M.	2 P. M.	2.10 P.
I	Temperature and result	102	104	
	Intravenous inoculation	5.0 c.c.		
	Treatment (intravenous)			
	Immune serum B			
	Blood culture			715,5

		1.30 P. M.
II	Temperature and result	102.2
	Intravenous inoculation	5.0 c.c.
	Untreated	
	Blood culture	

		10.30 A. M.	1 P. M.	1.1
III	Temperature and result	101.2	104.2	
	Intravenous inoculation	5.0 c.c.		
	Treatment (intravenous)			
	Normal serum			5
	Blood culture			8

		Mar.		
		11.30 A. M.	2 P. M.	2.15 P. M.
IV	Temperature and result	102.6	105.7	
	Intravenous inoculation	5.0 c.c.		
	Treatment (intravenous)			
	Immune serum B and A			*B*:5.0 c.c
	Blood culture			8,958,066

		12.30 P. M.	3
V	Temperature and result	102.2	103
	Intravenous inoculation	5.0 c.c.	
	Treatment (intravenous)		
	Immune serum B and A		*B*:5
	Blood culture		9,9

		12.30 P. M.	3 P. M
VI	Temperature and result	102.2	103.6
	Intravenous inoculation	5.0 c.c.	
	T reatment		
	Leucocyte extract		5.0 c.
	Blood culture		3,483,8

TABLE V.

Experiment of March 22, 1911, Page 95.

	Mar. 22.					Mar. 23.			Mar. 24.	Mar. 25.	Mar. 27.	Mar. 29.
	2.15 P. M.	3.15 P. M.	3.20 P. M.	3.25 P. M.	5.30 P. M.	8 A. M.	3 P. M.	5 P. M.				
		105			105.6	104	102.8	103	104.2	103.2	103	102.5 Cured.
	5.0 c.c.		62	5.0 c.c.	12			877				Sterile.

Mar. 25.		Mar. 26.
4.30 P. M.	10.30 P. M.	
104	101.3	Died in 18 hours.
1,408,468	1,424,640	

Mar. 28.			
P. M.	2.15 P. M.	4.20 P. M.	6 P. M.
	105.2	103.6	102.8. Died in 12 hours.
c.c.	5.0 c.c.		
,240	1,227,180	1,065,471	

	Apr. 1.			Apr. 2.		Apr. 3.			Apr. 4.		Apr. 5.		Apr. 6.
3 P. M.	5 P. M.	11 P. M.	8 A. M.	12 M.	5 P. M.	A. M.	P. M.	8 A. M.	4.30 P. M.	5.30 P. M.	A. M.	P. M.	2.30 P. M.	5 P. M.	
105.5	105	104.6	103.6	103.8	104.2	104	103	104.3	104.2	104.6	101.2	103.2	100	97	Died.
													Subcutaneous		
A:5.0 c.c. 128	106			12,397					*A*:5.0 c.c. 1,110	34,152			*A*:5.0 c.c. Sterile		Sterile.

Apr. 7.		
M.	4 P. M.	5.30 P. M.
3	104.2	104. Died in 18 hours.
c.c.	*A*:5.0 c.c.	
,520	3,739	2,567

Apr. 9.		
	4 P. M.	6 P. M.
	104	104.6. Died in 18 hours.
	Subcutaneous 5.0 c.c.	
27	6,900,600	12,363,840

normal rabbit serum, and the third with leucocyte extract prepared according to the method of Hiss, but of double strength. In the studies of Hiss and in several of my experiments with pneumococci of moderate virulence, this leucocyte extract materially modified the course of infection in the rabbit. It was more active than normal serum, and in the experiments of Hiss it was more efficient than immune serum. But owing to the excessively virulent inoculation in this series of experiments the leucocyte extract caused no modification in the course of the infection. Hence, with normal serum, leucocyte extract made an excellent control.

Experiment of March 22, 1911.—Three animals were treated with immune sera. The supply of the most active serum, designated B and obtained by immunization with living cultures, was limited, and at the less important points in the treatment another serum, A, derived from immunization with culture filtrates, and thus purely antitoxic in action, was substituted for it. That this other serum had to be employed was unfortunate from the point of view of securing complete cures, but was fortunate for purposes of comparison.

Treatment was given intravenously three hours after inoculation in the controls and in the test animals alike except in animal IV. In this instance, on account of the extreme prostration of the animal, it was unwise to wait longer than two and one half hours. Before each serum treatment and again two hours afterward blood cultures were taken in all animals. In the case of the untreated control, they were taken two hours after inoculation. This blood was also centrifugalized in citrate solution and the leucocytes were examined for signs of phagocytosis.

The records of the experiment appear in table V.

In the experiments recorded in table V the inoculation of 5 c.c. of the culture was excessive. In all the animals the effects of the poison were shown by immediate prostration, panting respiration, and very rapid heart action.

The controls, whether treated with normal serum, or with leucocyte extract, or left untreated, died in twelve to eighteen hours. The bacteriemia, as shown by the blood cultures, developed immediately after inoculation and increased rapidly. At autopsy the pneumococci were numerous in all of the tissues and organs. The severity of the toxemia was manifest from the degree of parenchymatous degeneration and from the extent of the hemorrhagic lesions present in all these animals.[7]

In the animals treated with immune sera the course of the infection was greatly modified. In all three, crises were induced by the immune sera; the prostration disappeared and the pneumococci of the bacteriemia were destroyed. Only one animal was completely cured. One died from thrombosis on the sixth day, but in the third animal the fatal issue was not delayed.

[7] Infections of the rabbit with many strains of pneumococci, even when they are fatal as early as these, are often free from hemorrhagic lesions and there is comparatively little evidence of severe poisoning.

The two immune sera differed greatly in the extent and possibly also in the character of their action. Serum B was obtained from rabbits highly immunized with living cultures; serum A, from rabbits immunized with culture filtrates and precipitates.

The treatment of animal I with serum B induced crisis: the bacteriemia fell in an hour from 715,500 cells per cubic centimeter to 62, and then in two hours to 12. The fever continued despite the marked visible improvement, but fell sharply to normal the following noon. Recrudescence with moderate increase of the bacteriemia proved evanescent, and thereafter the animal was normal.

Similarly in animal IV, serum B induced crisis. The bacteriemia fell in an hour from 8,959,060 cells per cubic centimeter to 128; then serum A was substituted, but caused no further reduction. As in animal I, the fever continued despite a most marked visible change in the dog (which was able to sit up and eat) but by the next day the fever fell sharply. Recrudescence with marked return of the bacteriemia developed the following day. On the third day serum A caused a marked fall in the temperature, but the bacteriemia increased from 1,110 to 34,142 in an hour. This must have been due to the thrombosed condition of the veins found later at autopsy. By the intravenous injection of serum the pneumococci were washed into the blood stream from a focus of infection. Subsequent serum treatment was given subcutaneously and the blood was sterile before and after its use. The animal's general condition improved steadily from the first treatment, but the ears became swollen and, without inflammatory reaction, the edema increased, involving the eye and cornea. The edema continued to increase, but the animal appeared well and ate as usual until the sixth day, when it died.

At autopsy a general thrombosis was found which extended from the ear vein through all the larger veins to the heart and through all the organs and extremities. No signs of toxemia or infection were present. Morphological examination of the blood and viscera failed to show any pneumococci. One c.c. of pericardial fluid was sterile, but from two drops of blood a few colonies of the pneumococcus developed, an evidence of the harmless parasitism which had persisted in this animal.

Animal V received only one dose of serum B, yet this reduced the bacteriemia in an hour from 9,902,520 to 3,739, and two hours later, after a dose of serum A, to 2,567. It is, however, doubtful if serum A had any significant action on the infection in this animal. In about eighteen hours the animal died.

At autopsy the lesions of toxemia and bacteriemia were even more marked than in the controls; especially in the heart in which the hemorrhagic lesions involved two thirds of the auriculo-ventricular ring, a third of the right auricle, and a part of the right ventricle. On morphological examination very few pneumococci were found and these were in the peritoneum. In culture, however, the pneumococcus was recovered from all the organs.

The study of the blood was especially significant in this experiment. In the three controls the development of the pneumococci proceeded unchecked, whereas in all three of the animals treated with immune sera the pneumococci were immediately destroyed in large numbers. Despite this, signs of phagocytosis were not found. When numerous, pneumococci were seen in the smears, but these were extracellular. The degenerative changes in the leucocytes were

marked in all the controls, and in animals IV and V at the second examination, but not in animal I at any stage of the infection. These changes were evanescent in animal IV, and were not present at subsequent examinations of the blood. Evidently the treatment with immune sera prevented the development of these changes in the leucocytes and also favored the recovery of the leucocytes.

Despite the remarkable destruction of the pneumococci in the first hour or two after treatment with this immune serum a few invariably survived. These persisted for several days as harmless parasites.[8] By the passive immunization with this serum treatment a condition was brought about which is comparable with that observed in the infection of animals actively immunized with culture filtrates. Clearly, in the recovery of the rabbit as in that of the dog, the neutralization of the infectious material was the dominant factor, the destruction of the bacteria being secondary.

SUMMARY OF THE STUDIES ON THE MECHANISM OF RECOVERY IN THE RABBIT.

From these experiments a clearer conception is gained of how certain phases of immunity modify the course of pneumococcus infection in the rabbit. The normal rabbit is so extremely susceptible to pneumococcus infection that the significance of the conditions of experiment and the effect of special treatment is clearly emphasized, a result not possible in experimenting on animals that are less susceptible to this infection and that possess normally a more complicated and efficient mechanism for recovery.

Phagocytosis played no part in the recovery of the rabbit from pneumococcus infection, yet crisis occurred spontaneously, and was induced experimentally by treatment with immune sera. In these crises there was a neutralization of the poison and a destruction of the bacteria which, according to modern conceptions of immunity,

[8] In the pleural infections of man similar conditions are not infrequently found. In the lung lesions it is perhaps less common, but it may persist for a long period as in Ewing's (6) case of unresolved pneumonia. Although the practical significance of this harmless parasitism is obvious it cannot be too strongly emphasized. Frequently this condition develops in animals immunized with living cultures and the danger of carrying over living pneumococci in serum therapy is a real one, against which the necessary precautions should be taken.

must be regarded as toxin and antitoxin neutralization and as bacteriolysis.

Bacteriolysis of the pneumococcus can be demonstrated *in vitro* and *in vivo,* but the presence of toxin is suggested only indirectly by the presence of substances which are separable from the bacterial cell and are capable of inducing an immunity in which protective substances neutralize the products of pneumococcus infection. This much seems to be definitely established, however narrowly we may limit our conceptions of toxin action.

The known toxins are recognized and measured only in terms of their action or their susceptibility to various influences. That toxin injures the body tissues is known. It is definitely recognized, moreover, that as they recover the injured tissues elaborate neutralizing antitoxins, and the amount of antitoxin formed may be precisely measured and demonstrated.

But it is not clear just how the tissues are poisoned. Toxin may act directly on the animal cell, as Ehrlich suggests; or, as now seems possible in the light of recent work on snake venin[9] and on anaphylaxis, toxin may bring about more complicated changes from which arise substances of manifold potency.

GENERAL CONSIDERATIONS.

There is no experimental evidence that lobar pneumonia in man differs fundamentally from pneumococcus infection in animals. Conditions of susceptibility vary in different animals and these determine the form and course of the disease. Conditions underlying recovery also vary and with them the manifestations of the protective mechanism. But fundamentally these conditions are similar.

As compared with the dog or with man, the rabbit is extremely susceptible to infection. Virulent pneumococci develop without local tissue reaction. The protective mechanism of the normal rabbit is slight and inadequate, and the animals die quickly from

[9] According to Dellezenne and Ledebt (7), constituents of cobra venin added to body fluids *in vitro* yield a substance 10,000 times as potent as the venin, thus suggesting that products of bacteria which form no toxin in the test-tube may give rise to very active poisons in the body.

the bacteriemia; but, when infection is prolonged, tissue adaptation develops rapidly and recovery may take place suddenly by crisis as in man. The sustained temperature of prolonged bacteriemia in the rabbit also resembles that of the lobar pneumonia of man.

Compared with the rabbit the dog is relatively insusceptible. Pneumococci develop and cause extensive local tissue reaction. The protective mechanism in the dog is efficient and infection fails to give rise to the sustained temperature, or to recovery by crisis comparable to that in man, despite the presence of characteristic and extensive lung lesions.

It may well be that in the lobar pneumonia of man the lung lesion plays a minor part in determining the disease as a whole, and that after all, even in man, it is the bacteriemia which accounts for many of the manifestations of the disease seen at the bedside. In both the dog and the rabbit, material that is infectious when inoculated under the skin or into a vein, is innocuous when injected into the lungs through the trachea. In all animals the protective mechanism of the lung is far more efficient than that of the blood stream.

In lobar pneumonia the extensive lesion is simply an expression of the efficiency of the individual's protective mechanism. This protective mechanism may be adequate or inadequate. That it is often adequate is shown by the early disappearance of the infectious agents in the exudate, not only in the experimental pneumonias of the dog and of the rabbit, but also in the lesions of fatal infections in man. Extensive lesions, however, are only incited by pneumococci of exalted virulence, and yet in the dog, and possibly also in man, the organisms must possess or acquire even greater virulence to induce the systemic manifestations of lobar pneumonia.

In man and in the dog extensive lung involvement may cause little discomfort, whereas serious symptoms often develop when the lesion of the lungs is comparatively slight. But apart from all this, the disease symptomatically is a systemic reaction to the poison, whether this poison be derived from the lung lesion or from the bacteriemia. For this reason, if pneumonia as it appears in man is to be investigated experimentally in animals, and if this study is to be complete, the bacteriemia of the rabbit and the pneumonic lesion of the dog must be studied.

Recovery of man or animals is due to the immunity incited. From the first, research has been directed in turn to one after another of the several phases of immunity, and in the course of this study attempts have been made to cure the disease by treatment with sera from immunized animals. But previous research was focussed on one phase of immunity and not on immunity as a whole; hence it failed to demonstrate satisfactorily the mechanism of recovery from pneumococcus infection, and the attempts to cure the disease by serum therapy failed to yield convincing results.

SUMMARY AND CONCLUSIONS.

From the results of this study of the action of immune sera on pneumococcus infection it is evident that immune sera vary greatly in their curative value. Immune sera possess protective action, but protective action is not necessarily indicative of curative action.

Treatment with the serum of normal rabbits may prolong the course of pneumococcus infection in the rabbit. This action, however, is slight and not always manifest. Sera from animals immunized with dead pneumococcus cells which had been washed free from their products, failed to exert materially greater curative action than normal sera. Sera from animals immunized with culture filtrates free from pneumococcus cells possessed, in some instances, a slight curative value, but often this curative action was not apparent.

In animals actively immunized, however, the presence of an immunity to culture filtrates was readily demonstrated. In the immunity produced by injections of dead culture material the strength was not sufficiently exalted for the sera to possess a practical curative value.

It was only after immunization with virulent living cultures that the blood serum acquired marked curative action. After pneumococcus infection in the rabbit had become established, treatment with this serum induced crisis and cured the animals.

From the results of the study of the mechanism of recovery it is evident that, despite the fact that virulent pneumococci are singularly insusceptible to the action of immune sera in the test-tube,

pneumococcus infection nevertheless conforms to the general law of infection.

Diphtheria and tetanus organisms give rise to powerful toxins, but the parasitism of these organisms is slight and their development is localized. Diseases produced by these organisms are toxemias and neutralization of their toxins by antitoxin puts an end to the disease.

The pneumococcus gives rise to toxic substances which are less active or are active only in the body tissues, but the parasitism of this organism is marked and its development is rarely localized. Nevertheless, the manifestations of the disease arise from the action of the bacterial poisons on the tissues. The neutralization of the pneumococcus poisons by immune serum puts an end to the symptoms of the disease, but the pneumococci survive as harmless parasites until destroyed by lysis or phagocytosis.

The neutralization of the pneumococcus poison may take place suddenly and completely as in crises; or, it may be incomplete with exacerbations of infection, as in lysis. Crisis, as it occurs in the lobar pneumonia of man and in the bacteriemia of the rabbit, is simply one phase of recovery, and recovery does not differ fundamentally, whether it is sudden and complete as in crisis, or incomplete and prolonged as in lysis, or whether the pneumococci are destroyed by lysis extracellularly as in the rabbit, or intracellularly as in the phagocytosis of the dog and man.

Since the recovery of animals from pneumococcus infection differs in no essential from that of man, since the unaided protective mechanism of man as compared with that of susceptible animals is exceptionally efficient, and since it is possible by treatment with sera from animals highly immunized with living cultures of virulent pneumococci to cure pneumococcus infection in the most susceptible animals, it is difficult to conceive of the infection in man failing to yield similarly to the administration of such sera.

BIBLIOGRAPHY.

1. Klemperer, G. and F., *Berl. klin. Wchnschr.*, 1891, xxviii, 833, 869.
2. Pane, N., *Centralbl. f. Bakteriol., Orig.*, 1879, xxi, 664.

3. Römer, P. H., Experimentelle und klinische Grundlagen für die Serumtherapie der Pneumokokkeninfektion der menschlichen Cornea (*Ulcus serpens*), Wiesbaden, 1909.
4. Neufeld, F., and Händel, *Zeitschr. f. Immunitätsforsch., Orig.*, 1909, iii, 159; *Arb. a. d. k. Gsndthtsamte.*, 1910, xxxiv, 166, 293.
5. Lamar, R. V., and Meltzer, S. J., *Jour. Exper. Med.*, 1912, xv, 133.
6. Ewing, J., *Proc. N. Y. Path. Soc.*, 1910, x, 90.
7. Dellezenne, C., and Ledebt, S., *Compt. rend. Acad. d. sc.*, 1911, cliii, 81; *Compt. rend. Soc. de biol.*, 1911, lxxi, 121.